Caspase Inhibitors and the Use Thereof

Background of the Invention

Field of the Invention

This invention is in the field of medicinal chemistry. In particular, the invention relates to dipeptide caspase inhibitors with novel N-terminal blocking groups. The invention also relates to the use of these caspase inhibitors for reducing or treating apoptotic cell death and/or reducing interleukin 1-β production.

Description of Background Art

Organisms eliminate unwanted cells by a process variously known as regulated cell death, programmed cell death or apoptosis. Such cell death occurs as a normal aspect of animal development as well as in tissue homeostasis and aging (Glucksmann, A., *Biol. Rev. Cambridge Philos. Soc. 26*:59-86 (1951); Glucksmann, A., *Archives de Biologie 76*:419-437 (1965); Ellis *et al., Dev. 112*:591-603 (1991); Vaux *et al., Cell 76*:777-779 (1994)). Apoptosis regulates cell number, facilitates morphogenesis, removes harmful or otherwise abnormal cells and eliminates cells that have already performed their function. Additionally, apoptosis occurs in response to various physiological stresses, such as hypoxia or ischemia (PCT published application WO96/20721).

There are a number of morphological changes shared by cells experiencing regulated cell death, including plasma and nuclear membrane blebbing, cell shrinkage (condensation of nucleoplasm and cytoplasm), organelle relocalization and compaction, chromatin condensation and production of apoptotic bodies (membrane enclosed particles containing intracellular material) (Orrenius, S., *J. Internal Medicine 237*:529-536 (1995)).

Apoptosis is achieved through an endogenous mechanism of cellular suicide (Wyllie, A. H., in *Cell Death in Biology and Pathology*, Bowen and

Lockshin, eds., Chapman and Hall (1981), pp. 9-34). A cell activates its internally encoded suicide program as a result of either internal or external signals. The suicide program is executed through the activation of a carefully regulated genetic program (Wylie *et al.*, *Int. Rev. Cyt. 68*:251 (1980); Ellis *et al.*, *Ann. Rev. Cell Bio. 7*:663 (1991)). Apoptotic cells and bodies are usually recognized and cleared by neighboring cells or macrophages before lysis. Because of this clearance mechanism, inflammation is not induced despite the clearance of great numbers of cells (Orrenius, S., *J. Internal Medicine 237*:529-536 (1995)).

Mammalian interleukin-1ß (IL-1ß) plays an important role in various pathologic processes, including chronic and acute inflammation and autoimmune diseases (Oppenheim *et. al. Immunology Today, 7*:45-56 (1986)). IL-1β is synthesized as a cell associated precursor polypeptide (pro-IL-1β) that is unable to bind IL-1 receptors and is biologically inactive (Mosley *et al., J. Biol. Chem. 262*:2941-2944 (1987)). By inhibiting conversion of precursor IL-1ß to mature IL-1ß, the activity of interleukin-1 can be inhibited. Interleukin-1ß converting enzyme (ICE) is a protease responsible for the activation of interleukin-1ß (IL-1ß) (Thornberry *et al.*, *Nature 356*:768 (1992); Yuan *et al.*, *Cell 75*:641 (1993)). ICE is a substrate-specific cysteine protease that cleaves the inactive prointerleukin-1 to produce the mature IL-1. The genes that encode for ICE and CPP32 are members of the mammalian ICE/Ced-3 family of genes which presently includes at least twelve members: ICE, CPP32/Yama/Apopain, mICE2, ICE4, ICH1, TX/ICH-2, MCH2, MCH3, MCH4, FLICE/MACH/MCH5, ICE-LAP6 and ICE_{rel}III. The proteolytic activity of this family of cysteine proteases, whose active site (a cysteine residue) is essential for ICE-mediated apoptosis, appears critical in mediating cell death (Miura *et al., Cell 75*:653-660 (1993)). This gene family has recently been named caspases (Alnemri *et. al. Cell, 87*:171 (1996), and Thornberry *et. al., J. Biol. Chem. 272*:17907-17911 (1997)) and divided into

three groups according to its known functions. Table 1 summarizes these known caspases.

Table I

Enzyme*
Group I: mediators of inflammation
Caspase-1 (ICE)
Caspase-4 (ICE_{rel}-II, TX, ICH-2)
Caspase-5 (ICE_{rel}-III, TY)
Group II: effectors of apoptosis
Caspase-2 (ICH-1, mNEDD2)
Caspase-3 (apopain, CPP-32, YAMA)
Caspase-7 (Mch-3, ICE-LAP3, CMH-1)
Group III: activators of apoptosis
Caspase-6 (Mch2)
Caspase-8 (MACH, FLICE, Mch5)
Caspase-9 (ICE-LAP6, Mch6)
Caspase-10

IL-1 is also a cytokine involved in mediating a wide range of biological responses including inflammation, septic shock, wound healing, hematopoiesis and growth of certain leukemias (Dinarello, C.A., *Blood 77*:1627-1652 (1991); diGiovine *et al., Immunology Today 11*:13 (1990)).

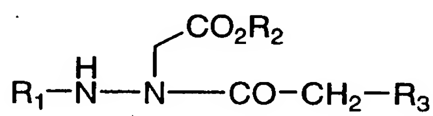
Many potent caspase inhibitors have been prepared based on the peptide substrate structures of caspases. However, in contrast to their potency *in vitro,* not too many inhibitors with good efficacy (IC_{50} < 1 μM) in whole-cell models of apoptosis have been reported (Thornberry, N. A. *Chem. Biol. 5:*R97-103 (1998)). Therefore the need exists for cell death inhibitors that are efficacy in whole-cell models of apoptosis and active in animal model of apoptosis. These inhibitors thus can be employed as therapeutic agents to treat disease states in which regulated cell death and the cytokine activity of IL-1 play a role.

WO 93/05071 discloses peptide ICE inhibitors with the formula:

$$Z-Q_2-\mathrm{Asp}-Q_1$$

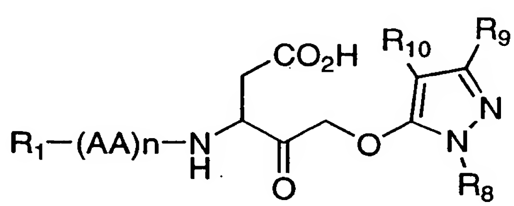
wherein Z is an N-terminal protecting group; Q_2 is 0 to 4 amino acids such that the sequence Q_2-Asp corresponds to at least a portion of the sequence Ala-Tyr-Val-His-Asp (SEQ ID NO:1); Q_1 comprises an electronegative leaving group.

WO 96/03982 discloses aspartic acid analogs as ICE inhibitors with the formula:



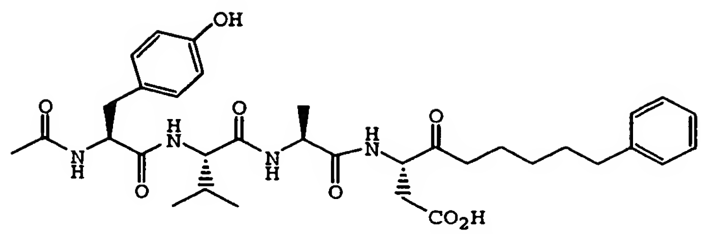
wherein R_2 is H or alkyl; R_3 is a leaving group such as halogen; R_1 is heteroaryl-CO or an amino acid residue.

U.S. patent 5,585,357 discloses peptidic ketones as ICE inhibitors with the formula:

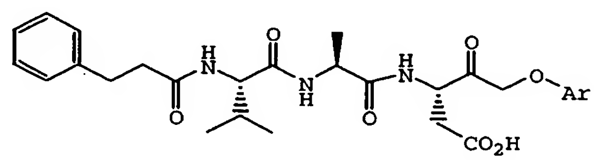


wherein n is 0-2; each AA is independently L-valine or L-alanine; R_1 is selected from the group consisting of N-benzyloxycarbonyl and other groups; R_8, R_9, R_{10} are each independently hydrogen, lower alkyl and other groups.

Mjalli *et al.* (*Bioorg. Med. Chem. Lett.* *3*:2689-2692 (1993)) report the preparation of peptide phenylalkyl ketones as reversible inhibitors of ICE, such as:

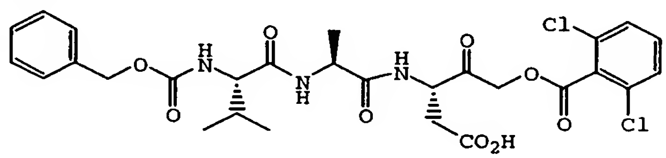


Thornberry *et al.* (*Biochemistry 33*:3934-3940 (1994)) report the irreversible inactivation of ICE by peptide acyloxymethyl ketones:

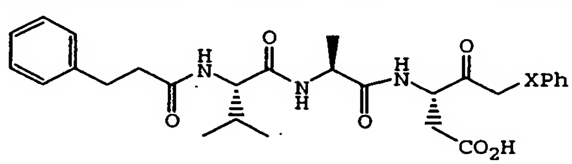


wherein Ar is COPh-2,6-$(CF_3)_2$, COPh-2,6-$(CH_3)_2$, Ph-F_5 and other groups.

Dolle *et al.* (*J. Med. Chem. 37*:563-564 (1994)) report the preparation of P_1 aspartate-based peptide α-((2,6-dichlorobenzoyl)oxy)methyl ketones as potent time-dependent inhibitors of ICE, such as:

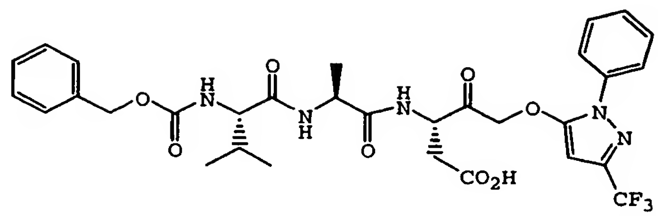


Mjalli *et al.* (*Bioorg. Med. Chem. Lett. 4*:1965-1968, (1994)) report the preparation of activated ketones as potent reversible inhibitors of ICE:

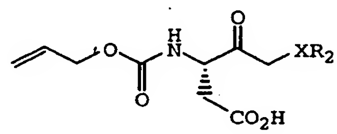


wherein X is $NH(CH_2)_2$, $OCO(CH_2)_2$, $S(CH_2)_3$ and other groups.

Dolle *et al.* (*J. Med. Chem. 37*:3863-3866 (1994)) report the preparation of α-((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy)methyl ketones as irreversible inhibitor of ICE, such as:

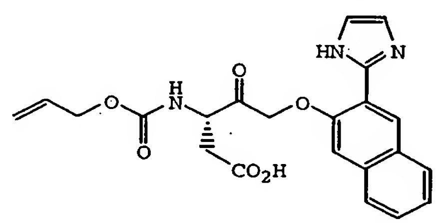


Mjalli *et al.* (*Bioorg. Med. Chem. Lett.* *5*:1405-1408 (1995)) report inhibition of ICE by N-acyl-Aspartic acid ketones:

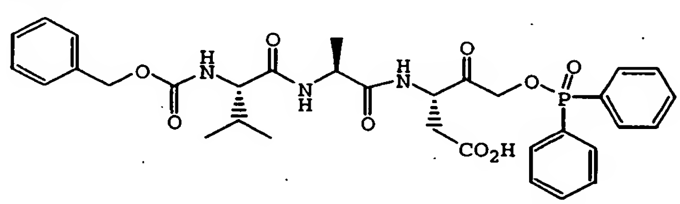


wherein XR_2 is $NH(CH_2)_2Ph$, $OCO(CH_2)_2$cyclohexyl and other groups.

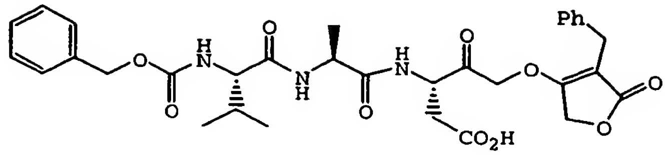
Mjalli *et al.* (*Bioorg. Med. Chem. Lett.* *5*:1409-1414 (1995)) report inhibition of ICE by N-acyl-aspartyl aryloxymethyl ketones, such as:

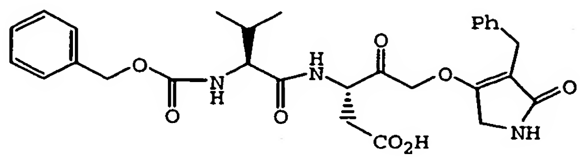


Dolle *et al.* (*J. Med. Chem.* *38*:220-222 (1995)) report the preparation of aspartyl α-((diphenylphosphinyl)oxy)methyl ketones as irreversible inhibitors of ICE, such as:

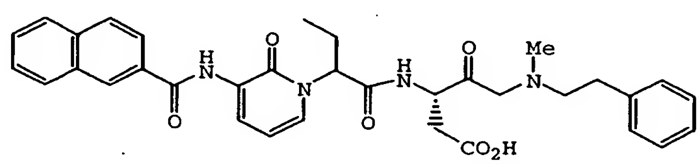


Graybill *et al.* (*Bioorg. Med. Chem. Lett.* *7*:41-46 (1997)) report the preparation of α-((tetronoyl)oxy)- and α-((tetramoyl)oxy)methyl ketones as inhibitors of ICE, such as:

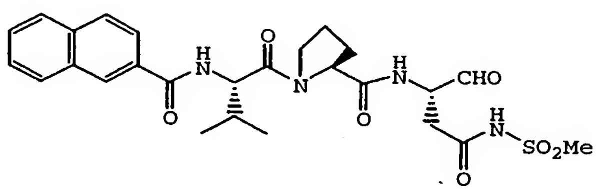




Semple *et al.* (*Bioorg. Med. Chem. Lett.* *8*:959-964 (1998)) report the preparation of peptidomimetic aminomethylene ketones as inhibitors of ICE, such as:



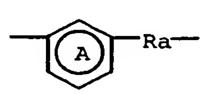
Okamoto *et al.* (*Chem. Pharm. Bull.* *47*:11-21 (1999)) report the preparation of peptide based ICE inhibitors with the P1 carboxyl group converted to an amide, such as:



EP618223 patent application discloses inhibitors of ICE as anti-inflammatory agents:

$$R\text{-}A_1\text{-}A_2\text{-}X\text{-}A_3$$

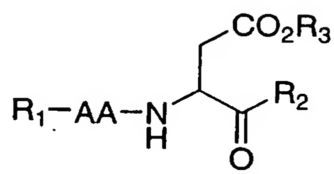
wherein R is a protecting group or optionally substituted benzyloxy; A_1 is an α-hydroxy or α-amino acid residue or a radical of formula:



wherein ring A is optionally substituted by hydroxy or $C_{1\text{-}4}$ alkoxy and R_a is CO or CS; A_2 is an α-hydroxy or α-amino acid residue or A_1 and A_2 form together a pseudo-dipeptide or a dipeptide mimetic residue; X is a residue

derived from Asp; A_3 is $-CH_2$-X_1-CO-Y_1, $-CH_2$-O-Y_2, $-CH_2$-S-Y_3, wherein X_1 is O or S; Y_1, Y_2 or Y_3 is cycloaliphatic residue, and optionally substituted aryl.

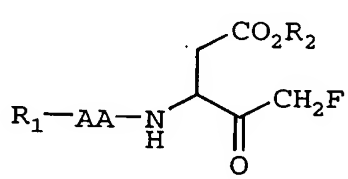
WO99/18781 discloses dipeptides of formula I:



I.

wherein R_1 is an N-terminal protecting group; AA is a residue of any natural or non-natural α-amino acid, β-amino acid, derivatives of an α-amino acid or β-amino acid; R_2 is H or CH_2R_4 where R_4 is an electronegative leaving group, and R_3 is alkyl or H, provided that AA is not His, Tyr, Pro or Phe. These dipeptides are surprisingly potent caspase inhibitors of apoptosis in cell based systems. These compounds are systemically active *in vivo* and are potent inhibitors of antiFas-induced lethality in a mouse liver apoptosis model and have robust neuroprotective effects in a rat model of ischemic stroke.

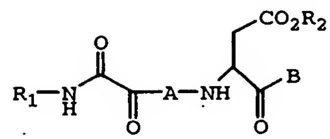
WO 99/47154 disclose dipeptides of formula I:



I

wherein R_1 is an N-terminal protecting group; AA is a residue of a non-natural α-amino acid or β-amino acid; R_2 is an optionally substituted alkyl or H.

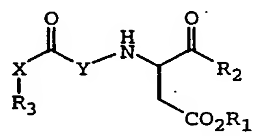
WO 00/01666 disclosed c-terminal modified oxamyl dipeptides as inhibitors of the ICE/ced-3 family of cysteine proteases:



wherein A is a natural or unnatural amino acid; B is a hydrogen atom, a deuterium atom, alkyl, cycloalkyl and other groups; R_1 is alkyl, cycloalkyl and other groups, R_2 is hydrogen, lower alkyl and other groups.

Summary of the Invention

The invention relates to compound of Formula I:



(I)

or pharmaceutically acceptable salts or prodrugs thereof, wherein:

R_1 is an optionally substituted alkyl or hydrogen;

R_2 is hydrogen or optionally substituted alkyl;

R_3 is an alkyl, saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heteroclyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted;

X is O, S, NR_4, or $(CR_4R_5)_n$, where R_4 and R_5 are, at each occurrence, independently selected from the group consisting of hydrogen, alkyl and cycloalkyl, and n is 0, 1, 2, or 3; or

X is NR_4, and R_3 and R_4 are taken together with the nitrogen atom to which they are attached to form a saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted; or

X is CR_4R_5, and R_3 and R_4 are taken together with the carbon atom to which they are attached to form a saturated carbocyclic, partially saturated

carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or oxygen-containing heteroaryl group, wherein said group is optionally substituted; and

Y is a residue of a natural or non-natural amino acid;

provided that when X is O, then R_3 is not unsubstituted benzyl or *t*-butyl; and when X is CH_2, then R_3 is not hydrogen.

The invention relates to the discovery that the compounds represented by Formula I are inhibitors of caspases. The invention also relates to the use of the compounds of the invention for reducing, preventing or treating maladies in which apoptotic cell death is either a causative factor or a result. Examples of uses for the present invention include protecting the nervous system following focal ischemia and global ischemia; treating neurodegenerative disorders such as Alzheimer's disease, Huntington's Disease, prion diseases, Parkinson's Disease, multiple sclerosis, amyotrophic lateral sclerosis, ataxia, telangiectasia, and spinobulbar atrophy; treating heart disease including myocardial infarction, congestive heart failure and cardiomyopathy; treating retinal disorders; treating autoimmune disorders including lupus erythematosus, rheumatoid arthritis, type I diabetes, Sjögren's syndrome and glomerulonephritis; treating polycystic kidney disease and anemia/erythropoiesis; treating immune system disorders, including AIDS and SCIDS; treating or ameliorating sepsis, reducing or preventing cell, tissue and organ damage during transplantation; reducing or preventing cell line death in industrial biotechnology; reducing or preventing alopecia (hair loss); and reducing the premature death of skin cells.

The present invention provides pharmaceutical compositions comprising a compound of Formula I in an effective amount to reduce apoptotic cell death in an animal.

The present invention also provides preservation or storage solutions for mammalian organs or tissue, or growth media for mammalian or yeast cells, wherein an effective amount of a compound of Formula I is included in

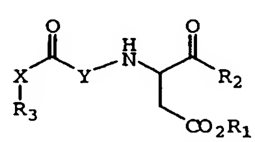
said solutions or media in order to reduce apoptotic cell death in said organs, tissue or cells.

The invention also relates to the use of caspase inhibitors for treating, ameliorating, and preventing non-cancer cell death during chemotherapy and radiation therapy and for treating and ameliorating the side effects of chemotherapy and radiation therapy of cancer.

In particular, the invention relates to a method of treating, ameliorating or preventing oral mucositis, gastrointestinal mucositis, bladder mucositis, proctitis, bone marrow cell death, skin cell death and hair loss resulting from chemotherapy or radiation therapy of cancer in an animal, comprising administering to the animal in need thereof an effective amount of a caspase inhibitor.

Detailed Description of the Invention

The inhibitors of caspases and apoptotic cell death of the present invention are compounds having the general Formula I:



(I)

or pharmaceutically acceptable salts or prodrugs thereof, wherein:

R_1 is an optionally substituted alkyl or hydrogen;

R_2 is hydrogen or optionally substituted alkyl;

R_3 is an alkyl, saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted;

X is O, S, NR_4, or $(CR_4R_5)_n$, where R_4 and R_5 are, at each occurrence, independently selected from the group consisting of hydrogen, alkyl and cycloalkyl, and n is 0, 1, 2, or 3; or

X is NR_4, and R_3 and R_4 are taken together with the nitrogen atom to which they are attached to form a saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted; or

X is CR_4R_5, and R_3 and R_4 are taken together with the carbon atom to which they are attached to form a saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or oxygen-containing heteroaryl group, wherein said group is optionally substituted; and

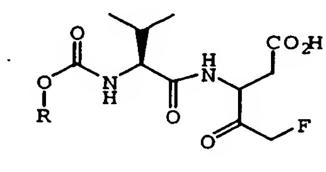
Y is a residue of a natural or non-natural amino acid;

provided that when X is O, then R_3 is not unsubstituted benzyl or *t*-butyl; and when X is CH_2, then R_3 is not hydrogen.

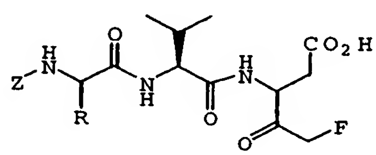
With respect to R_1, preferred alkyl groups are C_{1-6} alkyl groups, e.g., methyl, ethyl, propyl, isopropyl, isobutyl, pentyl and hexyl groups; and substituted C_{1-6} alkyl groups, e.g., CH_2OCH_3 and CH_2OCOCH_3 (AM or acetoxymethyl).

Preferred R_2 are alkyl group substituted by electronegative group or leaving group, including fluoromethyl, chloromethyl, alkoxymethyl, aryloxymethyl, alkylthiomethyl, arylthiomethyl, aminomethyl, acyloxymethyl, and arylacyloxymethyl. Other examples of optional substituents that may be present at the R_2 alkyl group include, without limitation, 3-pyrazolyloxy optionally substituted at the 2, 4 and 5-positions with lower alkyl; 3-(1-phenyl-3-trifluoromethyl)pyrazolyloxy; 2,6-di(trifluoromethyl)benzoyloxy; 2,6-dimethylbenzoyloxy; pentafluoro-phenoxy; tetrafluorophenoxy; 2,6-dichlorobenzoyloxy; 2-(3-(2-imidazolyl)naphthyl)oxy; diphenylphosphinyloxy; tetronyloxy; and tetramoyloxy.

The R_3 group in compounds of Formula I is designed to function as the P_3 side chain in a tripeptide. Structure A is an example of a dipeptide inhibitor of Formula I. In comparison, structure B is an example of a tripeptide inhibitor.



(A)



(B)

Preferred X is O, NH and CH_2. With respect to R_3, preferred alkyl are methyl, ethyl, isopropyl, isobutyl; preferred substituents on alkyl are hydroxy, carboxy, halogen, C_4-C_7 cycloalkyl, saturated and partially saturated heterocyclic, aryl or heteroaryl; preferred cycloalkyl are cyclopentyl and cyclohexyl; preferred saturated and partially saturated heterocyclic groups are piperidinyl and morpholinyl; preferred aryls are phenyl and naphthyl; preferred heteroaryls are pyridyl, indolyl, furyl and thienyl; preferred substituents in the aryl and heteroaryl are methyl, ethyl, chloro, fluoro, bromo, trifluoromethyl, methoxy, hydroxy, carboxy, cyano and nitro.

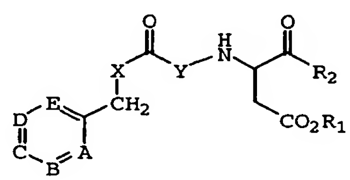
With respect to Y, preferred natural and non-natural amino acid are valine, isoleucine, leucine, proline, alanine, phenylalanine, methionine, serine, threonine, tryptophan, tyrosine, 2-aminobutyric acid, cyclohexylglycine, phenylglycine, cyclopentylglycine and t-butylglycine. Especially preferred amino acids are valine, isoleucine, leucine, alanine, phenylalanine, cyclohexylalanine, 2-aminobutyric acid, cyclohexylglycine, and phenylglycine.

The invention relates to the discovery that the compounds represented by Formula I are inhibitors of caspases. These inhibitors slow or block cell

death in a variety of clinical conditions and industrial applications in which the loss of cells, tissues or entire organs occurs. Therefore, the invention is also related to methods of treating, preventing or reducing conditions in which apoptosis plays a role. These conditions are more fully described below.

The methods comprise administering to an animal in need of such treatment an inhibitor of the present invention, or a pharmaceutically acceptable salt or prodrug thereof, in an amount effective to inhibit apoptotic cell death.

Another group of preferred embodiments of the present invention that may be employed as inhibitors of caspases are represented by Formula II:



(II)

or pharmaceutically acceptable salts or prodrugs thereof wherein R_1, R_2, X and Y are as defined previously with respect to Formula I; and

A is CR_6 or nitrogen;

B is CR_7 or nitrogen;

C is CR_8 or nitrogen;

D is CR_9 or nitrogen;

E is CR_{10} or nitrogen; provided that not more than three of A, B, C, D and E are nitrogen; and R_6-R_{10} independently are hydrogen, halo, C_1-C_6 haloalkyl, C_6-C_{10} aryl, C_4-C_7 cycloalkyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_6-C_{10} aryl(C_1-C_6)alkyl, C_6-C_{10} aryl(C_2-C_6)alkenyl, C_6-C_{10} aryl(C_2-C_6)alkynyl, C_1-C_6 hydroxyalkyl, nitro, amino, cyano, C_1-C_6 acylamino, hydroxy, C_1-C_6 acyloxy, C_1-C_6 alkoxy, alkylthio, or carboxy; or

one of R_6 and R_7, or R_7 and R_8, or R_8 and R_9, or R_9 and R_{10} are taken together with the carbon atoms to which they are attached to form a carbocycle or heterocycle.

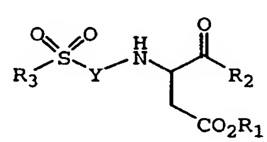
Examples of bridges formed by R_6 and R_7, or R_7 and R_8, or R_8 and R_9, or R_9 and R_{10} taken together are —OCH_2O—, —OCF_2O—, —$(CH_2)_3$—, —$(CH_2)_4$—, —OCH_2CH_2O—, —$CH_2N(R_{13})CH_2$—, —$CH_2CH_2N(R_{13})CH_2$—, —$CH_2N(R_{13})CH_2CH_2$—, –$N(R_{13})$–CH=CH–, –CH=CH–$N(R_{13})$–, -O-CH=CH–, –CH=CH–O–, –S–CH=CH–, –CH=CH–S–, –N=CH–CH=CH–, –CH=N–CH=CH–, –CH=CH–N=CH–, –CH=CH–CH=N–, -N=CH–CH=N–, and -CH=CH—CH=CH—;

where R_{13} is hydrogen, alkyl or cycloalkyl;

provided that when X is O, A is CR_6, B is CR_7, C is CR_8, D is CR_9 and E is CR_{10}, then at least one of the R_6-R_{10} is not a hydrogen.

Preferred R_1 is H, Me, Et, t-Bu or AM. Preferred R_2 is fluoromethyl, acyloxymethyl, arylacyloxymethyl, aryloxymethyl, phophinyloxymethyl or aminomethyl.

Another preferred group of the inhibitors of caspases and apoptotic cell death of the present invention are compounds having the general Formula III:



(III)

or pharmaceutically acceptable salts or prodrugs thereof wherein R_1, R_2, R_3 and Y are as defined previously with respect to Formula I.

Preferred R_1 is H, Me, Et, t-Bu or AM. Preferred R_2 is fluoromethyl, acyloxymethyl, arylacyloxymethyl, aryloxymethyl, phophinyloxymethyl or aminomethyl. Preferred R_3 is optionally substituted alkyl or aryl. Preferred Y

is valine, isoleucine, leucine, alanine, phenylalanine, cyclohexylalanine, 2-aminobutyric acid, cyclohexylglycine or phenylglycine.

Exemplary preferred inhibitors of caspases having Formular I include, without limitation:

2-Chlorobenzyloxycarbonyl-Val-Asp-fmk,
3-Chlorobenzyloxycarbonyl-Val-Asp-fmk,
4-Chlorobenzyloxycarbonyl-Val-Asp-fmk,
Phenethoxycarbonyl-Val-Asp-fmk,
Cyclohexylmethoxycarbonyl-Val-Asp-fmk,
Methoxycarbonyl-Val-Asp-fmk,
Ethoxycarbonyl-Val-Asp-fmk,
Isopropyloxycarbonyl-Val-Asp-fmk,
2-Chlorobenzyloxycarbonyl-Ile-Asp-fmk,
3-Chlorobenzyloxycarbonyl-Ile-Asp-fmk,
4-Chlorobenzyloxycarbonyl-Ile-Asp-fmk,
Phenylacetyl-Val-Asp-fmk,
4-Nitrobenzyloxycarbonyl-Val-Asp-fmk,
2,5-Dimethylbenzyloxycarbonyl-Val-Asp-fmk,
3,4-Dichlorobenzyloxycarbonyl-Val-Asp-fmk,
3,5-Dichlorobenzyloxycarbonyl-Val-Asp-fmk,
2,5-Dichlorobenzyloxycarbonyl-Val-Asp-fmk,
2,6-Dichlorobenzyloxycarbonyl-Val-Asp-fmk,
2,4-Dichlorobenzyloxycarbonyl-Val-Asp-fmk,
2,4-Dimethylbenzyloxycarbonyl-Val-Asp-fmk,
4-Ethylbenzyloxycarbonyl-Val-Asp-fmk,
4-Bromobenzyloxycarbonyl-Val-Asp-fmk,
4-Fluorobenzyloxycarbonyl-Val-Asp-fmk,
Cyclopentylmethoxycarbonyl-Val-Asp-fmk,
4-Trifluoromethylbenzyloxycarbonyl-Val-Asp-fmk,
3-Phenylpropionyl-Val-Asp-fmk,

Benzylaminocarbonyl-Val-Asp-fmk,
3-Phenylpropyloxycarbonyl-Val-Asp-fmk,
2,4-Difluorobenzyloxycarbonyl-Val-Asp-fmk,
3,4-Difluorobenzyloxycarbonyl-Val-Asp-fmk,
4-Morpholinecarbonyl-Val-Asp-fmk,
4-Pyridylmethoxycarbonyl-Val-Asp-fmk,
2-Pyridylmethoxycarbonyl-Val-Asp-fmk,
2,6-Dichlorobenzyloxycarbonyl-Val-Asp-DCB-methylketone,
Isobutoxycarbonyl-Val-Asp-fmk,
Propionyl-Val-Asp-fmk,
Benzyl-glutaryl-Val-Asp-fmk,Glutaryl-Val-Asp-fmk,
3-(2-Phenyloxyphenyl)propionyl-Val-Asp-fmk,
3-(5-Bromo-2-hydroxyphenyl)propionyl-Val-Asp-fmk,
3-Fluorobenzyloxycarbonyl-Val-Asp-fmk,
2-Fluorobenzyloxycarbonyl-Val-Asp-fmk,
3-Methylbenzyloxycarbonyl-Val-Asp-fmk,
2-Chloro-4-fluorobenzyloxycarbonyl-Val-Asp-fmk,
2-Naphthylmethoxycarbonyl-Val-Asp-fmk,
p-Toluenesulfonyl-Val-Asp-fmk, and
p-Toluenesulfonyl-Phe-Asp-fmk.

where fmk is fluoromethylketone and DCB is 2,6-dichlorobenzoyloxy.

Useful aryl groups are C_{6-14} aryl, especially C_{6-10} aryl. Typical C_{6-14} aryl groups include phenyl, naphthyl, phenanthrenyl, anthracenyl, indenyl, azulenyl, biphenyl, biphenylenyl and fluorenyl groups.

Useful cycloalkyl groups are C_{3-8} cycloalkyl. Typical cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

Useful saturated or partially saturated carbocyclic groups are cycloalkyl groups as defined above, as well as cycloalkenyl groups, such as cyclopentenyl, cycloheptenyl and cyclooctenyl.

Useful halo or halogen groups include fluorine, chlorine, bromine and iodine.

Useful alkyl groups include straight-chained and branched C_{1-10} alkyl groups, more preferably C_{1-6} alkyl groups. Typical C_{1-10} alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, *sec*-butyl, *tert*-butyl, 3-pentyl, hexyl and octyl groups. Also contemplated is a trimethylene group substituted on two adjoining positions on the benzene ring of the compounds of the invention.

Useful arylalkyl groups include any of the above-mentioned C_{1-10} alkyl groups substituted by any of the above-mentioned C_{6-14} aryl groups. Useful values include benzyl, phenethyl and naphthylmethyl.

Useful haloalkyl groups include C_{1-10} alkyl groups substituted by one or more fluorine, chlorine, bromine or iodine atoms, e.g., fluoromethyl, difluoromethyl, trifluoromethyl, pentafluoroethyl, 1,1-difluoroethyl, chloromethyl, chlorofluoromethyl and trichloromethyl groups.

Useful alkoxy groups include oxygen substituted by one of the C_{1-10} alkyl groups mentioned above.

Useful alkylthio groups include sulphur substituted by one of the C_{1-10} alkyl groups mentioned above. Also included are the sulfoxides and sulfones of such alkylthio groups.

Useful acylamino groups are any C_{1-6} acyl (alkanoyl) attached to an amino nitrogen, e.g., acetamido, propionamido, butanoylamido, pentanoylamido, hexanoylamido as well as aryl-substituted C_{2-6} substituted acyl groups.

Useful acyloxy groups are any C_{1-6} acyl (alkanoyl) attached to an oxy (-O-) group, e.g., formyloxy, acetoxy, propionoyloxy, butanoyloxy, pentanoyloxy, hexanoyloxy and the like.

Useful arylacyloxy groups include any of the aryl groups mentioned above substituted on any of the acyloxy groups mentioned above, e.g., 2,6-

dichlorobenzoyloxy, 2,6-difluorobenzoyloxy and 2,6-di-(trifluoromethyl)-benzoyloxy groups.

Useful amino groups include $-NH_2$, $-NHR_{11}$, and $-NR_{11}R_{12}$, wherein R_{11} and R_{12} are C_{1-10} alkyl or cycloalkyl groups as defined above.

Useful saturated or partially saturated heterocyclic groups include tetrahydrofuranyl, pyranyl, piperidinyl, piperizinyl, pyrrolidinyl, imidazolidinyl, imidazolinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, isochromanyl, chromanyl, pyrazolidinyl pyrazolinyl, tetronoyl and tetramoyl groups.

Useful heteroaryl groups include any one of the following: thienyl, benzo[b]thienyl, naphtho[2,3-b]thienyl, thianthrenyl, furyl, pyranyl, isobenzofuranyl, chromenyl, coumarinyl, xanthenyl, phenoxanthiinyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, phthalzinyl, naphthyridinyl, quinozalinyl, cinnolinyl, pteridinyl, carbazolyl, β-carbolinyl, phenanthridinyl, acrindinyl, perimidinyl, phenanthrolinyl, phenazinyl, isothiazolyl, phenothiazinyl, isoxazolyl, furazanyl, phenoxazinyl, 1,4-dihydroquinoxaline-2,3-dione, 7-aminoisocoumarin, pyrido[1,2-a]pyrimidin-4-one, 1,2-benzoisoxazol-3-yl, benzimidazolyl, 2-oxindolyl and 2-oxobenzimidazolyl. Where the heteroaryl group contains a nitrogen atom in a ring, such nitrogen atom may be in the form of an N-oxide, e.g., a pyridyl N-oxide, pyrazinyl N-oxide, pyrimidinyl N-oxide and the like.

Optional substituents include one or more alkyl; halo; haloalkyl; cycloalkyl; hydroxy; carboxy; phosphinyloxy; aryl optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl or heteroaryl groups; aryloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl or heteroaryl groups; aralkyl; heteroaryl optionally substituted with one or more lower alkyl, halo,

amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; heteroaryloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; alkoxy; alkylthio; arylthio; amino; alkylamino; dialkylamino; acyloxy; arylacyloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; diphenylphosphinyloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, or haloalkyl groups; heterocyclo optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; heterocycloalkyloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; partially saturated heterocycloalkyl optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups; or partially saturated heterocycloalkyloxy optionally substituted with one or more lower alkyl, halo, amino, alkylamino, dialkylamino, alkoxy, hydroxy, carboxy, haloalkyl and aryl groups.

Certain of the compounds of the present invention may exist as stereoisomers including optical isomers. The invention includes all stereoisomers and both the racemic mixtures of such stereoisomers as well as the individual enantiomers that may be separated according to methods that are well known to those of ordinary skill in the art.

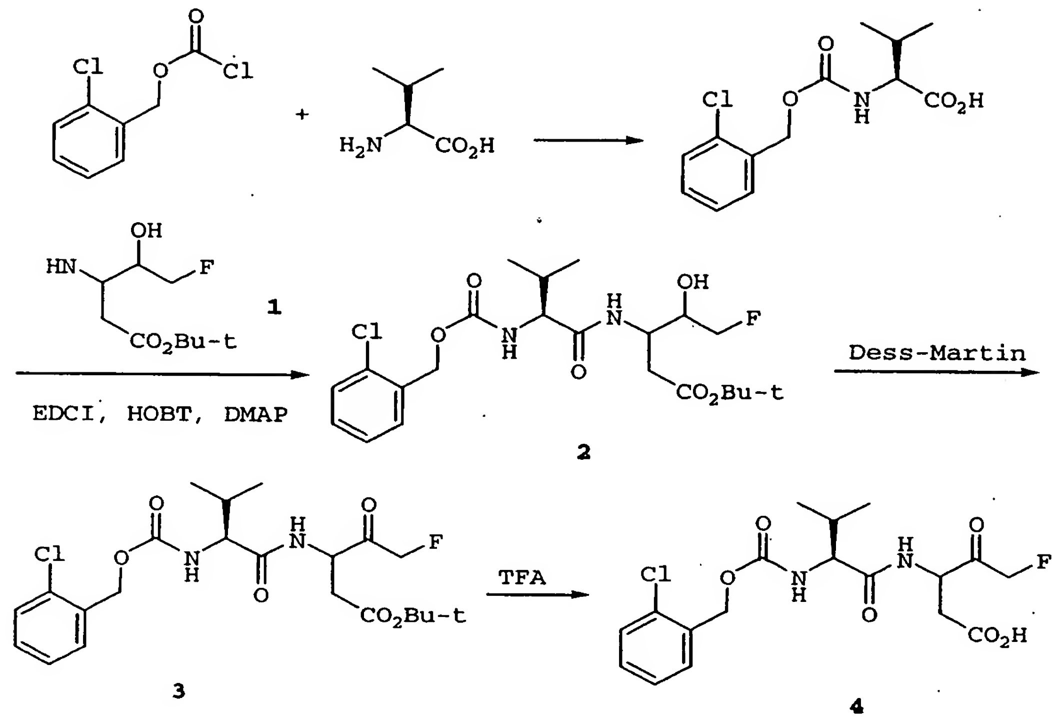
Examples of pharmaceutically acceptable addition salts include inorganic and organic acid addition salts such as hydrochloride, hydrobromide, phosphate, sulphate, citrate, lactate, tartrate, maleate, fumarate, mandelate and oxalate; and inorganic and organic base addition salts with bases such as sodium hydroxy and Tris(hydroxymethyl)aminomethane (TRIS, tromethane).

Examples of prodrugs include compounds of Formula I wherein R_1 is an alkyl group or substituted alkyl group such as CH_2OCH_3 and CH_2OCOCH_3 (AM ester).

The invention is also directed to a method for treating disorders responsive to the inhibition of caspases in animals suffering thereof. Particular preferred embodiments of compounds for use in the method of this invention are represented by previously defined Formula I.

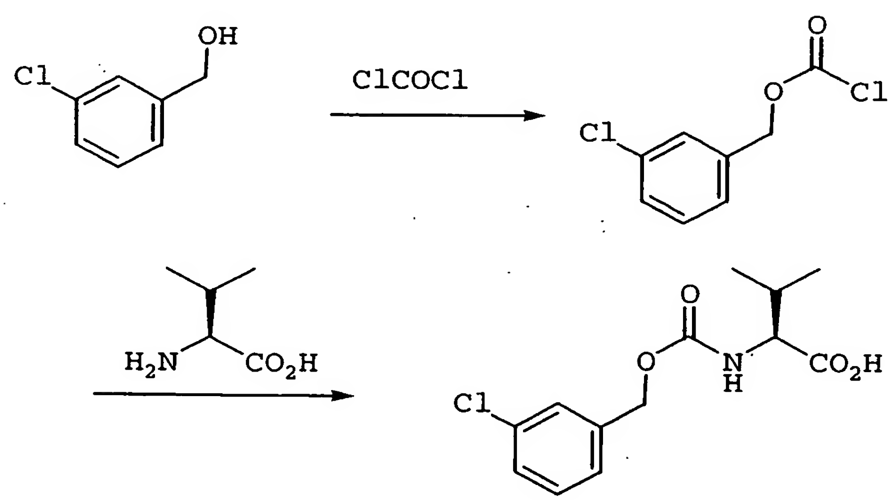
The compounds of this invention may be prepared using methods known to those skilled in the art. Specifically, compounds with Formula I can be prepared as illustrated by exemplary reactions in Scheme 1. The intermediate **1** was prepared according to Revesz *et al.* (*Tetrahedron Lett.* *35*:9693-9696 (1994)). Coupling of **1** with a N-protected amino acid, such as (2-chlorobenzyloxycarbonyl)-Val, which was prepared from 2-chlorobenzyl chloroformate and Valine, gave amide **2.** Oxidation of **2** by Dess-Martin reagent according to Revesz *et al.* (*Tetrahedron Lett.* *35*:9693-9696 (1994)) gave **3** as a mixture of diastereomers. The oxidation also can be done using other agents such as pyridinium chlorochromate (PCC) or pyridinium dichromate (PDC). Acid catalyzed cleavage of the ester gave the free acid **4.**

Scheme 1

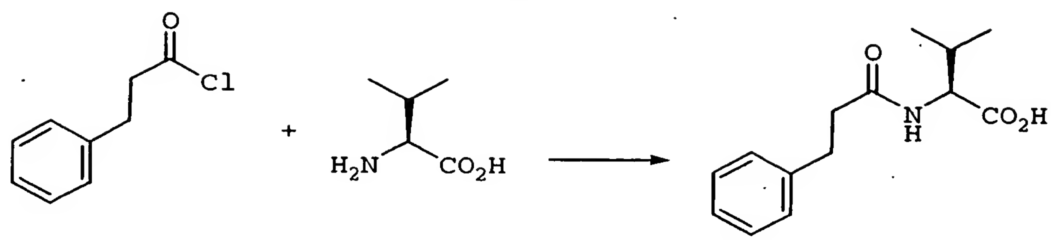


Other N-protected amino acid can be prepared as illustrated by exemplary reactions in Scheme 2-4.

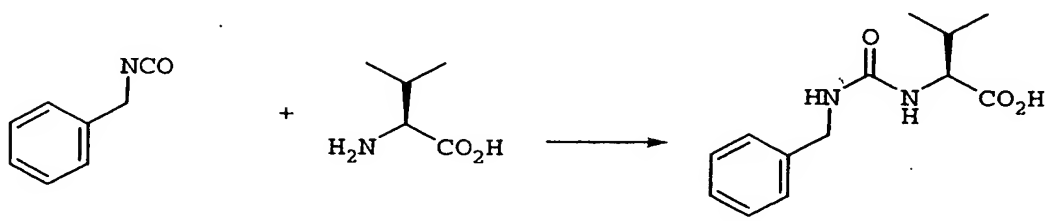
Scheme 2



Scheme 3

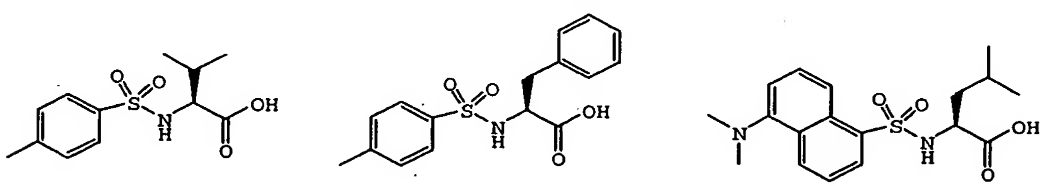


Scheme 4



Compounds of Formula III with substituted-sulfonyl as N-protecting group can be prepared similar to what described in Scheme 1. Examples of sulfonyl protected amino acids which can be used for the preparation of novel caspase inhibitors of Formula III are shown in Scheme 5.

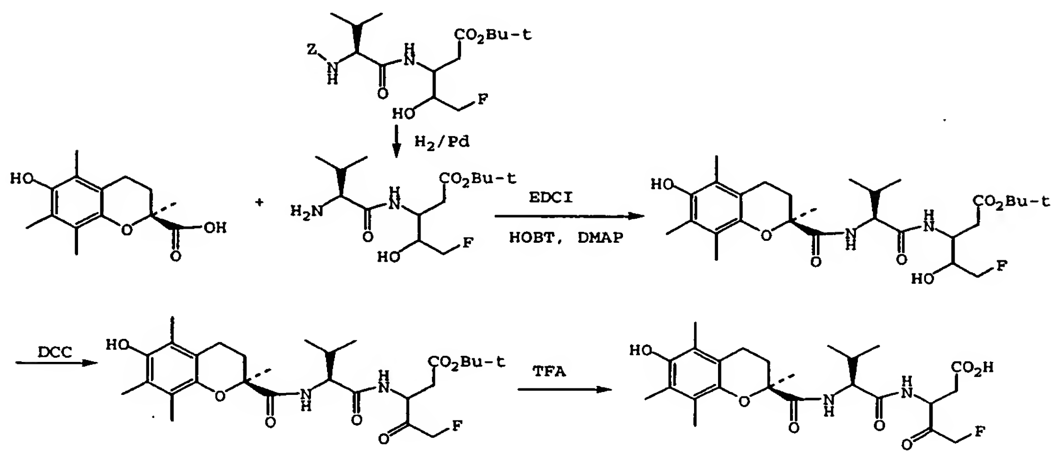
Scheme 5



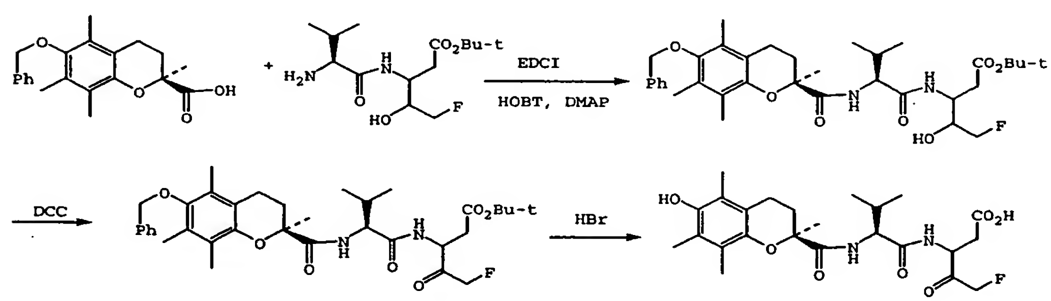
Other N-protecting group with special function also can be used. For instance, an antioxidant such as Trolox can be introduced as the protecting group. The compound can be prepared as shown in Scheme 6. Alternatively, the compound can be prepared as shown in Scheme 7. The compound will combine the property of a caspase inhibitor with an antioxidant, which might

be more efficacious as a neuroprotectant for the treatment of stroke (Pierre-Etienne Chabrier *et al.*, *PNAS 96*:10824-10829 (1999)).

Scheme 6

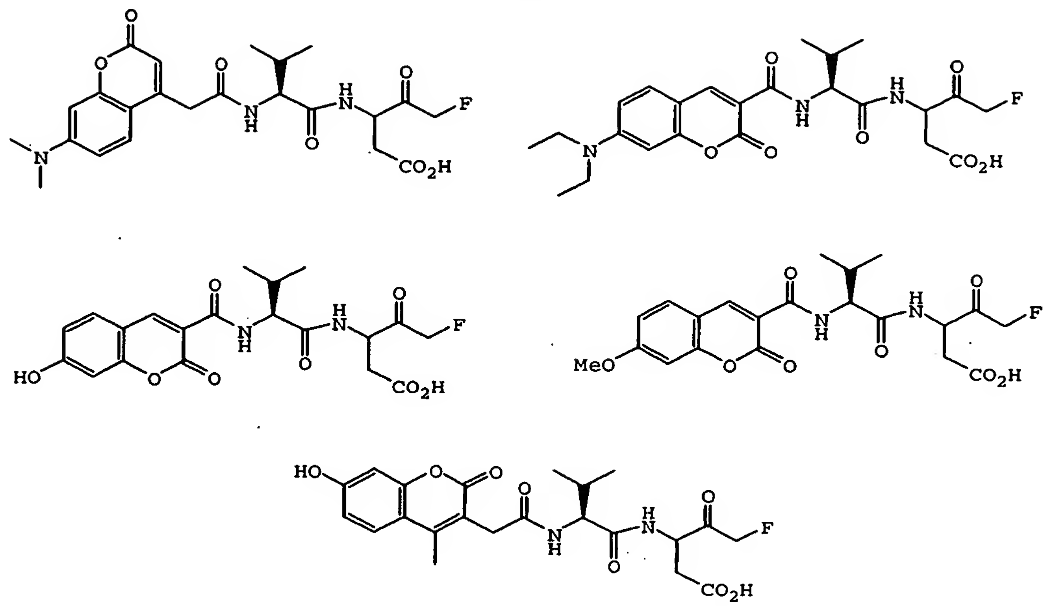


Scheme 7



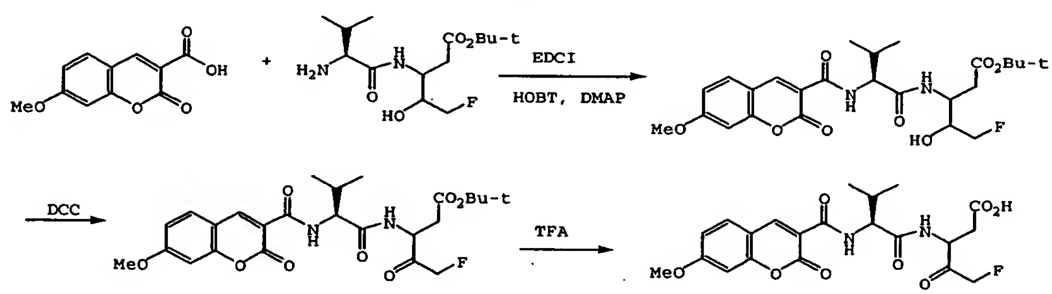
A fluorescent dye also can be introduced as the protecting group, such as the compounds shown in Scheme 8.

Scheme 8

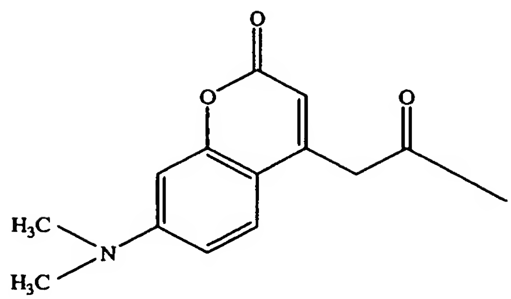


These compounds can inhibit caspase and resulted in the attachment of the fluorescent dye to the caspase. Therefore these molecules should be useful for the labeling of caspase and detection of caspase activity in the cells. These compounds can be prepared as illustrated by exemplary reactions in Scheme 9.

Scheme 9



Preferred fluorescent protecting groups of formula R_3–X–C(O)– include, for example:



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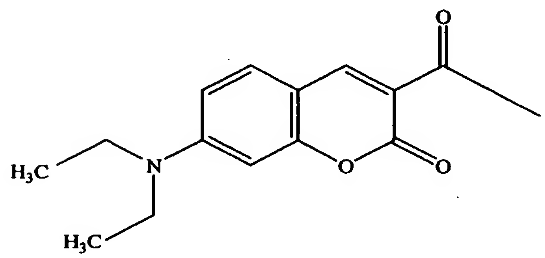

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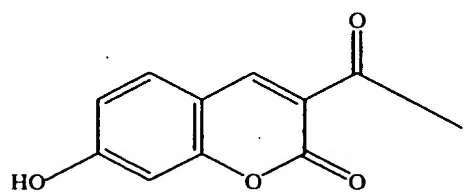

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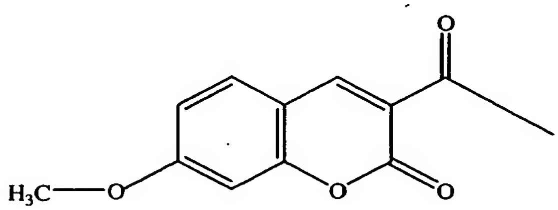
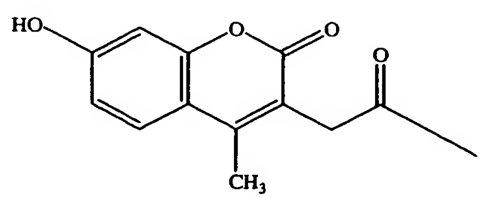


and



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An important aspect of the present invention is the discovery that compounds having Formula I are inhibitors of caspases. Therefore, these inhibitors are expected to slow or block cell death in a variety of clinical conditions in which the loss of cells, tissues or entire organs occurs.

The cell death inhibitors of the present invention can be used to reduce or prevent cell death in the nervous system (brain, spinal cord, and peripheral nervous system) under various conditions of ischemia and excitotoxicity, including, but not limited to, focal ischemia due to stroke and global ischemia due to cardiac arrest, as well as spinal cord injury (Emery *et al., J. Neurosurgery 89*:911-920 (1998)). One particular usage is to treat the effects of oxygen deprivation which can occur during the birth of infants in high-risk labors or drowning. The cell death inhibitors can also be used to reduce or prevent cell death in the nervous system due to traumatic injury (such as head trauma), viral infection or radiation-induced nerve cell death (for example, as a side-effect of cancer radiotherapy), as well as acute bacterial meningitis (Braun *et al., Nat Med* 5:298-302 (1999)). The cell death inhibitors can also be used to reduce or prevent cell death in a range of neurodegenerative disorders, including but not limited to Alzheimer's disease (Mattson *et al., Brain Res. 807*:167-176 (1998)), Huntington's Disease, Parkinson's Disease, multiple sclerosis, amyotrophic lateral sclerosis, and spinobulbar atrophy. The *in vivo* neuroprotective properties of cell death inhibitors of the invention can be tested in a rat transient focal brain ischemia model (Xue *et al., Stroke 21*:166 (1990)). The cell death inhibitors may also be used to treat or ameliorate cell death in acute bacterial meningitis (Braun *et al., Nat Med* 5:298-302 (1999))

The cell death inhibitors of the invention can be used to reduce or prevent cell death in any condition which potentially results in the death of cardiac muscle (Black *et al., J. Mol. Cel. Card. 30*:733-742 (1998) and Maulik *et al., Free Radic. Biol. Med. 24*:869-875 (1998)). This includes myocardial infarction due to myocardial ischemia and reperfusion, congestive heart failure

and cardiomyopathy. One particular application is to reduce or prevent myocardial cell death as occurs in certain viral infections of the heart.

The *in vivo* activity of the cell death inhibitors of the invention can be tested using the "mouse liver apoptosis" model described by Rodriguez *et al.* (*J. Exp. Med. 184*:2067-2072 (1996)). In this model, mice are treated intravenously (IV) with an antiFas antibody which induces massive apoptosis in the liver and other organs, leading to generalized organ failure and death. This model is useful for indirectly testing the systemic bioavailability of the cell death inhibitors of the invention, as well as their *in vivo* anti-apoptotic properties. The cell death inhibitors of the invention therefore can be used to reduce or prevent apoptosis of liver cells (Jones *et al., Hepatology 27*:1632-42 (1998)) such as in sepsis (Jaeschke *et al., J. Immunol. 160*:3480-3486 (1998)) and hereditary tyrosinemia type 1 (HT1) (Kubo *et al., Prov. Natl. Acad. Sci. USA 95*:9552-9557 (1998)). The cell death inhibitors of the invention also can be used to treat hepatitis (Suzuki, *Proc. Soc. Exp. Biol. Med. 217*:450-454 (1998)).

The cell death inhibitors of the invention can be used to reduce or prevent cell death of retinal neurons (Kermer *et al., J. Neurosci. 18*:4656-4662 (1998) and Miller *et al., Am. J. Vet. Res. 59*:149-152 (1998)) as can occur in disorders which increase intraocular pressure (such as glaucoma) or retinal disorders associated with the aging process (such as age-related macular degeneration). The inhibitors can also be used to treat hereditary degenerative disorders of the retina, such as retinitis pigmentosa.

The cell death inhibitors of the invention can also be used to reduce or prevent cell death in the kidney. This includes renal amyloidosis (Hiraoka *et al., Nippon Jinzo Gakkai Shi. 40*:276-83 (1998)), acute renal failure (Lieberthal *et al., Semin Nephrol. 18*:505-518 (1998)), murine tubular epithelial cell death induced by cyclosporine A (Ortiz *et al., Kidney International Supp. 68*:S25-S29 (1998)) and HIV-induced nephropathy (Conaldi *et al., J. Clin. Invest. 102*:2041-2049 (1998)).

The cell death inhibitors of the invention can also be used to reduce or prevent cell death of buccal mucosa due to chronic alcohol ingestion (Slomiany *et al., Biochem. Mol. Biol. Int. 45*:1199-1209 (1998)).

The cell death inhibitors of the invention can also be used to reduce or prevent cell death in plants (Richberg *et al., Curr. Opin. Plant Biol. 1*:480-485 (1998)), such as plant cell death due to pathogens (Pozo *et al., Curr. Biol. 8*:1129-1132 (1998) and Greenberg *et al., Cell 77*:551-563 (1994)).

The cell death inhibitors of the invention can also be used to reduce or prevent cell death due to radiation and ultraviolet-irradiation (Sheikh *et al., Oncogene 17*:2555-2563 (1998)).

The cell death inhibitors of the invention can also be used to reduce or prevent apoptotic death of bone marrow cells in myelodysplastic syndromes (MDS) (Mundle *et al., Am. J. Hematol. 60*:36-47 (1999)).

The cell death inhibitors of the invention can also be used to reduce or prevent premature death of cells of the immune system, and are particularly useful in treating immune deficiency disorders, such as acquired immune deficiency syndrome (AIDS), severe combined immune deficiency syndrome (SCIDS) and related diseases. The cell death inhibitors can also be used to treat radiation-induced immune suppression.

Transplantation of human organs and tissues is a common treatment for organ failure. However, during the transplantation process, the donor organ or tissue is at risk for cell death since it is deprived of its normal blood supply prior to being implanted in the host. This ischemic state can be treated with cell death inhibitors by infusion into the donor organ or tissue, or by direct addition of the cell death inhibitors to the organ/tissue storage medium. For example, it was reported that treatment of the embryonic nigral cell suspension with Ac-YVAD-cmk (SEQ ID NO:2), a caspase-1 inhibitor, mitigated DNA fragmentation and reduced apoptosis in transplants. It also increased survival of dopaminergic neurons grafted to hemiparkinsonian rats, and thereby substantially improved functional recovery (Schierle *et al., Nat. Med. 5*:97-100

(1999)). Cell death inhibitors may also be used to reduce or prevent cell death in the donor organ/tissue after it has been transplanted to protect it from the effects of reperfusion injury and /or effects of host immune cells which kill their targets by triggering apoptosis. The cytoprotective effects of cell death inhibitors can also be used to prevent the death of human or animal sperm and eggs used in *in vitro* fertilization procedures. These inhibitors can be used during the harvesting process and can also be included in the storage medium.

Mammalian cell lines, insect cells and yeast cells are commonly used to produce large amounts of recombinant proteins (such as antibodies, enzymes or hormones) for industrial or medicinal use. The lifespan of some of these cell lines is limited due to growth conditions, the nature of the recombinant molecule being expressed (some are toxic) and other unknown factors. The lifespans of industrial cell lines can be extended by including these cell death inhibitors in the growth medium in a concentration range of 1-100 μM.

The factors governing hair growth and loss are largely unknown. There is some evidence, however, that hair follicle regression (referred to as catagen) may be due at least partially to apoptosis. Therefore, it is contemplated that the cell death inhibitors of the present invention can be used to treat hair loss that occurs due to various conditions, including but not limited to male-pattern baldness, radiation-induced or chemotherapy-induced hair loss, and hair loss due to emotional stress. There is also evidence that apoptosis may play a role in the loss of hair color. Therefore, it is contemplated that the cell death inhibitors of the present invention can also be used in treating or preventing cases of premature graying of the hair.

The death of skin epithelial cells can occur after exposure to high levels of radiation, heat or chemicals. It is contemplated that the cell death inhibitors of the present invention can be used to treat, reduce or prevent this type of skin damage. In one particular application, the cell death inhibitors can

be applied as part of a topical formulation, e.g., an ointment, to treat acute over-exposure to the sun and to prevent blistering and peeling of the skin.

Goldberg *et al.* (*Nature Genetics 13*: 442-449 (1996)) reported recently that huntingtin, a protein product of Huntington's disease (HD) gene, can be cleaved by CPP32 but not ICE. The mutation underlying HD is an expansion of a CAG trinucleotide at the 5′ end of the HD gene. The trinucleotide expansion exceeding 36 repeats is associated with the clinical presentation of HD. The CAG expansion promotes cleavage of huntingtin by CPP32, thus links the role of CPP32 in the apoptotic cell death in HD. Compounds of the present invention with CPP32 inhibiting activity will be useful in blocking CPP32 induced apoptotic cell death, thus in preventing and treating HD and other disorders characterized by expansion of trinucleotide repeats such as myotonic dystrophy, fragile X mental retardation, spinobulbar muscular atrophy, spinocerebellar atoxia type I and Dentato-Rubro pallidoluysian atrophy.

The invention relates to a method of treating, ameliorating or preventing oral mucositis, gastrointestinal mucositis, bladder mucositis, proctitis, bone marrow cell death, skin cell death and hair loss resulting from chemotherapy or radiation therapy of cancer in an animal, comprising administering to the animal in need thereof an effective amount of a cell death inhibitor of the present invention.

When animals are treated with chemotherapeutic agents and/or radiation to kill cancer cells, an unwanted side effect is the apoptotic death of rapidly dividing non-cancer cells. Such non-cancer cells include cells of the gastrointestinal tract, skin, hair, and bone marrow cells. According to the present invention, caspase inhibitors are administered to such non-cancer cells to prevent apoptosis of such cells. In a preferred embodiment, the caspase inhibitors are administered locally, e.g., to the gastrointestinal tract, mouth, skin or scalp to prevent apoptosis of the gastrointestinal, mouth, skin or hair cells but allowing for the death of the cancer cells. Thus, in one example, it is

possible to treat brain cancer with chemotherapy or radiation therapy and protect the outer skin, hair cells, gastrointestinal tract and bone marrow by local administration of a caspase inhibitor. In the case of oral mucositis, the caspase inhibitor can be applied, for example, in the form of a mouth wash or mouth rinse, in a gel, or in the form of an oral slow release lozenge to prevent activation of caspases and apoptotic cell death induced by the chemotherapeutic agent or by radiation. In the case of gastrointestinal mucositis, the caspase inhibitor can be applied in a form such that it is not absorbed systemically or in a form that coats the surface of the gastrointestinal tract, or a suppository formulation for the treatment of gastrointestinal mucositis. In the case of proctitis, the capsase inhibitor may be applied as part of an enema or suppository. In the case of bladder mucositis, the caspase inhibitor may be applied though a bladder catheter. For prevention of radiation or chemotherapy-induced hair loss, the caspase inhibitor can be applied, for example, to the scalp in the form of a hair rinse, hair gel, shampoo or hair conditioner. Importantly, the caspase inhibitor can be applied prior to the administration of the chemotherapeutic agent or radiation, thus preventing the onset of the damaging effects of the chemotherapeutic agent or radiation to the normal cells.

Compositions within the scope of this invention include all compositions wherein the compounds of the present invention are contained in an amount which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typically, the compounds may be administered to mammals, e.g., humans, orally at a dose of 0.0025 to 50 mg/kg, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated for apoptosis-mediated disorders, e.g., neuronal cell death, heart disease, retinal disorders, polycystic kidney disease, immune system disorders and sepsis. Preferably, about 0.01 to about 10 mg/kg is orally administered to treat or prevent such

disorders. For intramuscular injection, the dose is generally about one-half of the oral dose. For example, for treatment or prevention of neuronal cell death, a suitable intramuscular dose would be about 0.0025 to about 25 mg/kg, and most preferably, from about 0.01 to about 5 mg/kg.

The unit oral dose may comprise from about 0.01 to about 50 mg, preferably about 0.1 to about 10 mg of the compound. The unit dose may be administered one or more times daily as one or more tablets each containing from about 0.1 to about 10, conveniently about 0.25 to 50 mg of the compound or its solvates.

In a topical formulation, the compound may be present at a concentration of about 0.01 to 100 mg per gram of carrier. In a preferred embodiment, the compound is present at a concentration of about 0.07-1.0 mg/ml, more preferably, about 0.1-0.5 mg/ml, most preferably, about 0.4 mg/ml.

In addition to administering the compound as a raw chemical, the compounds of the invention may be administered as part of a pharmaceutical preparation containing suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those preparations which can be administered orally or topically and which can be used for the preferred type of administration, such as tablets, dragees, slow release lozenges and capsules, mouth rinses and mouth washes, gels, liquid suspensions, hair rinses, hair gels, shampoos and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection, topically or orally, contain from about 0.01 to 99 percent, preferably from about 0.25 to 75 percent of active compound(s), together with the excipient.

Also included within the scope of the present invention are the non-toxic pharmaceutically acceptable salts of the compounds of the present invention. Acid addition salts are formed by mixing a solution of the

particular cell death inhibitors of the present invention with a solution of a pharmaceutically acceptable non-toxic acid such as hydrochloric acid, fumaric acid, maleic acid, succinic acid, acetic acid, citric acid, tartaric acid, carbonic acid, phosphoric acid, oxalic acid, and the like. Basic salts are formed by mixing a solution of the particular cell death inhibitors of the present invention with a solution of a pharmaceutically acceptable non-toxic base such as sodium hydroxide, potassium hydroxide, choline hydroxide, sodium carbonate Tris and the like.

The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compounds of the invention. Foremost among such animals are mammals, e.g., humans, although the invention is not intended to be so limited.

The caspase inhibitors and pharmaceutical compositions thereof may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, buccal, intrathecal, intracranial, intranasal or topical routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. In general, the caspase inhibitors are administered locally to the tissues that are to be protected from apoptosis and separately from the chemotherapeutic agent. For example, cisplatin may be administered by i.v. injection to treat a cancer such as brain, lung, breast, liver, kidney, pancreatic, ovarian, prostatic cancer, and the caspase inhibitor administered locally to treat, ameliorate, or prevent apototic cell death in the mouth or gastrointestinal tract, such as a mouth wash for the treatment of oral mucositis; and IV injectable aqueous solution for the treatment of bone marrow cell death; and an oral formulation suitable for coating the gastrointestinal surfaces or an emema or suppository formulation for the treatment of gastrointestinal mucositis including proctitis. The caspase

inhibitors may also be applied through a bladder catheter for the treatment, amelioration or prevention of bladder mucositis. Alternatively or concurrently, the caspase inhibitors may be applied topically to the skin and/or scalp to treat, ameliorate or prevent apoptotic cell death of hair and skin cells. In a further embodiment, the chemotherapeutic agent or radiation may be applied locally to treat a localized cancer such as brain, lung, breast, liver, kidney, pancreatic, ovarian, prostatic cancer, and the caspase inhibitor administered systemically, e.g., by i.v. injection, to treat, ameliorate or prevent apoptotic cell death of the gastrointestinal tract cells, mouth epithelial cells, bone marrow cells, skin cells and hair cells. In the case of oral mucositis in brain cancer treatment, for example, a caspase inhibitor that does not penetrate the blood-brain barrier can be applied, for example, systemically by i.v. injection followed by irradiation of the brain tumor. This would protect the oral mucosa from the harmful effects of radiation but the caspase inhibitor would not protect the brain tumor from the therapeutic effects of radiation. Importantly, the caspase inhibitor can be applied prior to administration of the radiation, thus preventing the onset of the damaging effects of the radiation to the normal mucosa cells.

The pharmaceutical preparations of the present invention are manufactured in a manner which is itself known, for example, by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding the resulting mixture and processing the mixture of granules, after adding suitable auxiliaries, if desired or necessary, to obtain tablets or dragee cores.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl

cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, above all, flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropymethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, enemas or suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal

capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts and alkaline solutions. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides or polyethylene glycol-400 (the compounds are soluble in PEG-400). Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

In accordance with one aspect of the present invention, compounds of the invention are employed in topical and parenteral formulations and are used for the treatment of skin damage, such as that caused by exposure to high levels of radiation, including ultraviolet radiation, heat or chemicals.

One or more additional substances which have therapeutic effects on the skin may also be incorporated in the compositions. Thus, the composition may also contain one or more compounds capable of increasing cyclic-AMP levels in the skin. Suitable compounds include adenosine or a nucleic acid hydrolysate in an amount of about 0.1-1% and papaverine, in an amount of about 0.5-5%, both by weight based on the weight of the composition. Also suitable are β-adrenergic agonists such as isoproterenol, in an amount of about 0.1-2% or cyclic-AMP, in an amount of about 0.1-1%, again both by weight based on the weight of the composition. Other suitable types of additional active ingredients which may be incorporated in the compositions of this invention include any compounds known to have a beneficial effect on skin. Such compounds include retinoids such as Vitamin A, in an amount of about 0.003-0.3% by weight and chromanols such as Vitamin E or a derivative

thereof in an amount of about 0.1-10% by weight, both based on the weight of the composition. Additionally, anti-inflammatory agents and keratoplastic agents may be incorporated in the cosmetic composition. A typical anti-inflammatory agent is a corticosteroid such as hydrocortisone or its acetate in an amount of about 0.25-5% by weight, or a corticosteroid such as dexamethasone in an amount of about 0.025-0.5% by weight, both based on the weight of the composition. A typical keratoplastic agent is coal tar in an amount of about 0.1-20% or anthralin in an amount of about 0.05-2% by weight, both based on the weight of the composition.

The topical compositions of this invention are formulated preferably as oils, creams, lotions, ointments and the like by choice of appropriate carriers. Suitable carriers include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohol (greater than C_{12}). The preferred carriers are those in which the active ingredient is soluble. Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Additionally, transdermal penetration enhancers can be employed in these topical formulations. Examples of such enhancers can be found in U.S. Patent Nos. 3,989,816 and 4,444,762.

Creams are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount of an oil such as almond oil, is admixed. A typical example of such a cream is one which includes about 40 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil.

Ointments may be formulated by mixing a solution of the active ingredient in a vegetable oil such as almond oil with warm soft paraffin and allowing the mixture to cool. A typical example of such an ointment is one which includes about 30% almond oil and about 70% white soft paraffin by weight.

Lotions may be conveniently prepared by dissolving the active ingredient, in a suitable high molecular weight alcohol such as propylene glycol or polyethylene glycol.

In addition, these compositions may include other medicinal agents, growth factors, wound sealants, carriers, etc., that are known or apparent to those skilled in the art. The compositions of the invention are administered to a warm-blooded animal, such as human, already suffering from a skin damage, such as a burn, in an amount sufficient to allow the healing process to proceed more quickly than if the host were not treated. Amounts effective for this use will depend on the severity of the skin damage and the general state of health of the patient being treated. Maintenance dosages over a prolonged period of time may be adjusted as necessary. For veterinary uses, higher levels may be administered as necessary.

In the case of an animal suffering from decreased hair growth, the compositions of the invention are administered in an amount sufficient to increase the rate of hair growth. Amounts effective for this use will depend on the extent of decreased hair growth, and the general state of health of the patient being treated. Maintenance dosages over a prolonged period of time may be adjusted as necessary. For veterinary uses, higher levels may be administered as necessary.

When the compounds are to be administered to plants, they may be applied to the leaves and/or stems and/or flowers of the plant, e.g., by spraying. The compounds may be spayed in particulate form or dissolved or suspended in an appropriate carrier, e.g., in water or an oil-water emulsion. The compounds may also be combined with the soil of the plant. In this embodiment, the compounds are taken up by the roots of the plant.

In a preferred embodiment, the caspase inhibitor is formulated as part of a mouthwash for the treatment, amelioration or prevention of oral mucositis. Such mouthwashes are aqueous solutions of the caspase inhibitor which may also contain alcohol, glycerin, synthetic sweeteners and surface-

active, flavoring and coloring agents. They may also contain anti-infective agents such as hexetidine and cetylpyridinium chloride. The mouthwashes may also contain topical anesthetics (e.g., benzocaine, cocaine, dyclonine hydrochloride, lidocaine, proparacaine hydrochloride or teracaine hydrochloride), for example, for relieving pain of radiation or chemotherapy-induced sores. The mouth washes may have either acidic or basic pH. See *Remington's Pharmaceutical Sciences*, A.R. Gennaro (ed.), Mack Publishing Company, pp. 1045, 1046, 1526 and 1965 (1990).

In another preferred embodiment, the caspase inhibitor is formulated as an oral formulation which is capable of coating the gastrointestinal surfaces for the treatment, amelioration or prevention of gastrointestinal mucositis. Examples of gastrointestinal mucositis include esophageal mucositis, gastric mucositis, and intestinal mucositis. Such formulations may comprise gastric antacids such as aluminum carbonate, aluminum hydroxide gel, bismuth subnitrate, bismuth subsalicylate, calcium carbonate, dihydroxyaluminum sodium carbonate, magaldrate, magnesium carbonate, magnesium hydroxide, magnesium oxide, sodium bicarbonate, milk of bismuth, dihydroxyaluminum aminoacetate, magnesium phosphate, magnesium trisilicate and mixtures thereof. Other additives include without limitation H_2-receptor antagonists, digestants, anti-emetics, adsorbants, and miscellaneous agents. See *Remington's Pharmaceutical Sciences*, A.R. Gennaro (ed.), Mack Publishing Company, pp. 774-778 (1990).

Chemotherapy agents such as cisplatin and radiation therapy often induce early and late onset emesis in the patient. Thus, in one embodiment an antiemetic is coadminstered together with the caspase inhibitor to avoid emesis and retain contact of the caspase inhibitor with the gastrointestinal tract. Examples of such antiemetics include without limitation compounds that block the dopaminergic emetic receptors such as metoclopramide and trimethobenzamide, and cannabinoids. Metoclopramide may be administered orally prior to and/or during chemotherapy/radiation therapy/caspase inhibitor

therapy to prevent the early emesis response and then later by intranasal administration according to U.S. Patent Nos. 5,760,086 and 4,536,386 to prevent delayed onset emesis. During the period after chemotherapy/radiation therapy, both the caspase inhibitor and the antiemetic may be coadministered to treat, ameliorate or prevent gastrointestinal mucositis.

In a further embodiment, the caspase inhibitor may be formulated as an IV injectable solution for the treatment, amelioration or prevention of bone marrow cell death.

The compositions of the invention may be administered to a warm-blooded animal, such as human, already suffering from chemotherapy or radiation therapy-induced non-cancer cell death, or, more preferably, before or during therapy with chemotherapy or radiation.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

Example 1

2-Chlorobenzyloxycarbonyl-Val-Asp-fmk

Step A. *2-Chlorobenzyl chloroformate.* To a solution of 2-chlorobenzyl alcohol (1.0 g, 7.0 mmol) in diethyl ether (15 ml) at 0°C was added *N,N*-diisopropylethyl amine (2.4 ml, 14.0 mmol), and phosgene solution in toluene (7.5 ml, 14.0 mmol). The mixture was allowed to warm up to room temperature in 2 h while stirring, then it was filtered. The diethyl ether was removed by rotary evaporator, and the solution of 2-chlorobenzyl chloroformate in toluene was carried on for the next step reaction.

Step B. *2-Chlorobenzyloxycarbonyl-Val.* To a solution of L-valine (0.5 g, 4.3 mmol) in 2 N NaOH aqueous solution (10 ml) was added 2-

chlorobenzyl chloroformate (14.0 mmol) at room temperature. The resulting solution was stirred at room temperature for 12 h, and then was diluted with 20 ml of ethyl acetate, washed with 2N NaOH, 2N HCl and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained as white solid (0.94 g, 3.29 mmol, 77%). ^{1}H NMR (DMSO-d_6): 12.24 (bs, 1 H), 7.61 (d, J = 8.4, 1 H), 7.50 (m, 2 H), 7.37 (m, 2 H), 5.12 (s, 2 H), 3.87 (dd, J = 8.7, 6.0, 1 H), 2.06 (m, 1 H), 0.89 (m, 6 H).

Step C. *t-Butyl 5-fluoro-3-(2-chlorobenzyloxycarbonyl-valinamido)-4-hydroxypentanoate.* To a solution of 2-chlorobenzyl-oxycarbonyl-Val (216 mg, 0.76 mmol) in THF (10 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (159 mg, 0.83 mmol), 1-hydroxybenzotriazole hydrate (HOBT) (116 mg, 0.77 mmol) and 4-(dimethylamino)pyridine (DMAP) (46 mg, 0.38 mmol). The resulting mixture was stirred at room temperature for 5 min, to which was then added a solution of *t*-butyl 3-amino-5-fluoro-4-hydroxypentanoate (157 mg, 0.75 mmol) in THF (5 ml). The resulting mixture was stirred at room temperature for 12 h, and diluted with ethyl acetate (20 ml), washed with 1N HCl, saturated $NaHCO_3$, brine and dried over Na_2SO_4. Evaporation of solvent, followed by flash chromatography (EtOAc/Hexane 2/3) gave the title compound as a colorless oil (80 mg, 0.17 mmol, 22%). ^{1}H NMR ($CDCl_3$): 7.38 (m, 2 H), 7.27 (m, 2 H), 7.06-6.84 (m, 1 H), 5.56 (m, 1 H), 5.22 (s, 2 H), 4.51-4.21 (m, 3 H), 4.01 (m, 3 H), 2.63 (m, 2 H), 2.12 (m, 1 H), 1.43 (m, 9H), 0.96 (m, 6H).

Step D. *2-Chlorobenzyloxycarbonyl-Val-Asp(OBu-t)-fmk.* To a suspension of Dess-Martin periodinane (0.35 g, 0.835 mmol) in dichloromethane (15 ml) was added a solution of *t*-butyl 5-fluoro-3-(2-chlorobenzyloxycarbonyl-valinamido)-4-hydroxypentanoate (80 mg, 0.17 mmol) in dichloromethane (5 ml). The mixture was refluxed for 12 h, cooled to room temperature, then it was diluted with 25 ml of ethyl acetate, washed with saturated Na_2SO_3 aqueous solution, brine, and then dried over Na_2SO_4. Evaporation of the solvent, followed by flash chromatography (EtOAc/Hexane

1/2) gave the title compound as a pale white solid (56 mg, 0.12 mmol, 72%). ^{1}H NMR ($CDCl_3$): 7.39 (m, 2 H), 7.28 (m, 2 H), 5.43 (m, 1 H), 5.25 – 4.84 (m, 5 H), 4.05 (m, 1 H), 2.96 (m, 1 H), 2.77 (m, 1 H), 2.14 (m, 1 H), 1.42 (s, 9H), 0.96 (m, 6H).

Step E. *2-Chlorobenzyloxycarbonyl-Val-Asp-fmk.* To a solution of 2-chlorobenzyloxycarbonyl-Val-Asp(OBu-*t*)-fmk (56 mg, 0.12 mmol) in 3 ml of CH_2Cl_2 at room temperature was added 1 ml of TFA. The resulting solution was allowed to stir for 3 h, and then diluted with 20 ml of ethyl acetate, washed with saturated Na_2HPO_4, brine, and dried over Na_2SO_4. The solvent was evaporated *in vacuo* to give the title compound as a white solid (39 mg, 0.09 mmol, 77%). ^{1}H NMR ($CDCl_3$): 8.61 (m, 1 H), 8.15 (m, 1 H), 7.49 (m, 2 H), 7.37 (m, 2 H), 5.21 (m, 1 H), 5.11 (s, 2 H), 4.59 (m, 2 H), 3.84 (m, 1 H), 2.65 (m, 2 H), 1.98 (m, 1H), 0.86 (m, 6 H).

Example 2

3-Chlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3-chlorobenzyl alcohol. ^{1}H NMR ($CDCl_3$): 7.55 (bs, 1 H), 5.48 (m, 1 H), 4.90 (m, 4 H), 4.02 (m, 2 H), 3.85 (m, 1 H), 3.08 (m, 1 H), 2.76 (m, 1 H), 1.93 (m, 1 H), 1.71 (m, 7 H), 1.23 (m, 4 H), 0.95 (m, 6 H).

Example 3

Phenethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from phenethyl alcohol. ^{1}H NMR ($CDCl_3$): 7.87 (m, 2 H), 7.23 (m, 5 H), 5.74 (d, J = 8.4, 1 H), 4.87 (m, 2 H), 4.26 (m, 3 H), 3.99 (m, 1 H), 2.89 (m, 4 H), 2.09 (m, 1 H), 0.91 (m, 6 H).

Example 4

4-Chlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 4-chlorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.51 (m, 1 H), 7.42 (m, 5 H), 5.21 (m, 1 H), 5.03 (s, 2 H), 4.60 (m, 1 H), 3.83 (m, 1 H), 2.65 (m, 2 H), 1.93 (m, 1 H), 0.85 (m, 6 H).

Example 5

Cyclohexylmethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from cyclohexylmethanol. ^{1}H NMR ($CDCl_3$): 7.55 (bs, 1 H), 5.48 (m, 1 H), 4.90 (m, 4 H), 4.02 (m, 2 H), 3.85 (m, 1 H), 3.08 (m, 1 H), 2.76 (m, 1 H), 1.93 (m, 1 H), 1.71 (m, 7 H), 1.23 (m, 4 H), 0.95 (m, 6 H).

Example 6

Ethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 1 from L-valine and ethyl chloroformate. ^{1}H NMR (DMSO-d_6): 8.43 (s, 1H), 7.19 (m, 1H), 5.14(bs, 2H), 4.66-4.53(m, 1H), 4.01(q, J=6.9, 2H), 3.83-3.76 (m, 1H), 2.73-2.67 (m, 2H), 1.96-1.88 (m, 1H), 1.16 (t, J=6.9, 3H), 0.86-0.82 (m, 6H).

Example 7

Benzylcarbonyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 1 from phenylacetyl chloride. ^{1}H NMR (DMSO-d_6): 8.55 (m, 1H), 8.21 (m,

1H), 7.21 (m, 5H), 5.14 (m, 2H), 4.62 (m, 2H), 4.14 (m, 1H), 2.66 (m, 2H), 1.93 (m, 1H), 0.83 (m, 6H).

Example 8
4-Nitrobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 1 from 4-nitrobenzyl chloroformate. ^{1}H NMR (DMSO-d_6): 12.5 (s, 1H), 8.66-8.54 (m, 1H), 8.23 (m, 2H), 7.64 (m, 2H), 5.19 (m, 4H), 4.66-4.54 (m, 2H), 3.82 (m, 1H), 2.76 (m, 2H), 1.95 (m, 1H), 0.87 (m, 6H).

Example 9
2,5-Dimethylbenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,5-dimethylbenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.50 (m, 1H), 7.42 (m, 1H), 7.06 (m, 2H), 4.99 (s, 2H), 4.64-4.56 (m, 1H), 3.83 (m, 1H), 2.97 (m, 1H), 2.67 (m, 2H), 1.89 (m, 1H), 0.86 (m, 6H).

Example 10
3,4-Dichlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3,4-dichlorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.50 (m, 1H), 7.65-7.35 (m, 4H), 5.03 (m, 3H), 4.59 (m, 1H), 3.84 (m, 1H), 2.67 (m, 2H), 1.94 (m, 1H), 0.86 (m, 6H).

Example 11

3,5-Dichlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3,5-dichlorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.54 (m, 1H), 7.56-7.34 (m, 3H), 5.05 (m, 3H), 4.63-4.55 (m, 1H), 3.86 (m, 1H), 2.73 (m, 2H), 1.95 (m, 1H), 0.86 (m, 6H).

Example 12

2,5-Dichlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,5-dichlorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.54 (m, 1H), 7.51 (m, 3H), 5.26-5.08 (m, 3H), 4.65-4.55 (m, 1H), 3.87 (m, 1H), 2.73-2.60 (m, 2H), 1.98 (m, 1H), 0.86 (m, 6H).

Example 13

2,6-Dichlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,6-dichlorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.50 (m, 1H), 7.55-7.41 (m, 3H), 5.25 (m, 3H), 4.64-4.51 (m, 1H), 3.78 (m, 1H), 2.71 (m, 2H), 1.92 (m, 1H), 0.84 (m, 6H).

Example 14

2,4-Dimethylbenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,4-dimethylbenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.53 (m, 1H), 7.39 (m, 1H), 7.28-7.18 (m, 3H), 5.26 (m, 1H), 4.99 (m, 2H), 4.66-4.53 (m, 1H),

3.80 (m, 1H), 2.78-2.72 (m, 1H), 2.59 (m, 3H), 1.92 (m, 1H), 1.16 (m, 3H), 0.85 (m, 6H).

Example 15

4-Ethylbenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 4-ethylbenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.50 (m, 1H), 7.23 (m, 4H), 4.98 (m, 3H), 4.63 (m, 1H), 3.84 (m, 1H), 2.68 (m, 2H), 1.91 (m, 1H), 0.85 (m, 6H).

Example 16

4-Chlorobenzyloxycarbonyl-Ile-Asp-fmk

The title compound was prepared in five steps as described in example 1 from 4-chlorobenzyl alcohol. ^{1}H NMR ($CDCl_3$): 8.61 (m, 1H), 8.52 (m, 1H), 7.48 (m, 1H), 7.39 (m, 4H), 5.19 (m, 2H), 5.02 (s, 2H), 4.54 (m, 1H), 3.87 (m, 1H), 2.73 (m, 2H), 1.68 (m, 1H), 1.39 (m, 1H), 1.17 (m, 1H), 0.81 (m, 6H).

Example 17

4-Bromobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in example 1 from 4-bromobenzyl alcohol. ^{1}H NMR (DMSO-*d6*): 7.56 (d, J = 8.1 Hz, 1H), 7.42 (m, 1H), 7.32 (d, J = 8.1 Hz, 1H), 5.21 (m, 1H), 5.00 (s, 2H), 4.62 (m, 2H), 3.83 (m, 1H), 3.60 (m, 1H), 2.71 (m, 2H), 1.76 (m, 1H), 0.84 (m, 6H).

Example 18

4-Fluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 4-fluorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.63 (m, 1H), 7.92 (m, 1H), 7.55 (m, 4H), 5.03 (m, 3H), 4.61 (m, 2H), 3.83 (m, 1H), 2.73 (m, 2H), 1.95 (m, 1H), 0.95 (m, 6H).

Example 19

2,4-Dichlorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,4-dichlorobenzyl alcohol. ^{1}H NMR ($CDCl_3$): 8.51 (m, 1H), 7.52 (m, 3H), 5.08 (m, 3H), 4.59 (m, 2H), 3.85 (m, 1H), 2.69 (m, 2H), 1.94 (m, 1H), 0.95 (m, 6H).

Example 20

Cyclopentylmethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from cyclopentylmethanol. ^{1}H NMR (DMSO-d_6): 7.22 (bs, 1H), 5.21 (m, 1H), 5.09 (m, 1H), 4.61 (m, 2H), 3.84 (m, 3H), 2.73 (m, 2H), 2.10 (m, 1H), 1.92 (m, 1H), 1.49 (m, 6H), 1.23 (m, 2H), 0.84 (m, 6H).

Example 21

4-Trifluoromethylbenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 1 from 4-trifluoromethylbenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.63 (m, 1H),

8.18 (m, 1H), 7.74 (d, J = 7.2 Hz, 2H), 7.57(d, J = 8.1, 2H), 5.14 (m, 4H), 4.64 (m, 1H), 3.81 (m, 1H), 2.72 (m, 2H), 1.94 (m, 1H), 0.86 (m, 6H).

Example 22

3-Phenylpropionyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 2 from 3-phenylpropionyl chloride. ^{1}H NMR (DMSO-d_6): 12.29 (bs, 1H), 8.39 (m, 1H), 7.82 (m, 1H), 7.21(m, 5H), 5.25 (m, 2H), 4.39 (m, 1H), 4.03 (m, 1H), 2.80 (m, 3H), 2.54 (m, 3H), 1.89 (m, 1H), 0.80 (m, 6H).

Example 23

Benzylaminocarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from benzyl amine. ^{1}H NMR (DMSO-d_6): 8.55 (m, 1H), 7.25 (m, 5H), 6.57 (m, 1H), 6.20 (m, 1H), 5.16 (m, 1H), 4.22 (m, 5H), 2.61 (m, 2H), 1.89 (m, 1H), 0.87 (m, 6H).

Example 24

3-Phenylpropyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3-phenyl-1-propanol. ^{1}H NMR (DMSO-d_6): 8.61 (m, 1H), 8.18 (m, 1H), 7.21 (m, 5H), 5.17 (m, 1H), 4.53 (m, 1H), 3.83 (m, 4H), 2.71 (m, 4H), 1.85 (m, 3H), 0.85 (m, 6H).

Example 25

2,4-Difluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2,4-difluorobenzyl alcohol. ^{1}H NMR ($CDCl_3$): 7.33 (m, 1H), 6.83 (m, 2H), 5.60 (m, 1H), 5.11 (m, 3H), 4.88 (m, 2H), 4.03 (m, 1H), 3.05 (m, 1H), 2.79 (m, 1H), 2.05 (m, 1H), 0.94 (m, 6H).

Example 26

3,4-Difluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3,4-difluorobenzyl alcohol. ^{1}H NMR (CDCl3): 8.63 (m, 1H), 8.18 (m, 1H), 7.52 (m, 1H), 7.27 (m, 2H), 5.07 (m, 3H), 4.51 (m, 2H), 3.99 (m, 1H), 2.70 (m, 2H), 1.95 (m, 1H), 0.85 (m, 6H).

Example 27

4-Morpholinecarbonyl-Val-Asp-fmk

The title compound was prepared in four steps as described in Example 1 from 4-morpholinecarbonyl chloride. ^{1}H NMR ($CDCl_3$): 10.61 (bs, 1H), 7.58 (m, 1H), 5.19 (d, J = 8.1 Hz, 1H), 4.95 (m, 3H), 4.42 (m, 1H), 3.71 (m, 4H), 3.42 (m, 4H), 2.94 (m, 2H), 2.19 (m, 1H), 0.98 (m, 6H).

Example 28

4-Pyridylmethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 4-pyridylcarbinol. ^{1}H NMR (CDCl3): 8.56 (m, 2H), 8.15 (m, 2H), 7.23

(m, 1H), 6.47 (m, 1H), 5.10 (s, 2H), 4.35 (m, 3H), 4.08 (m, 1H), 2.61 (m, 2H), 1.46 (m, 1H), 0.94 (m, 6H).

Example 29

2-Pyridylmethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2-pyridylcarbinol. ^{1}H NMR ($CDCl_3$): 8.59 (m, 1H), 7.28 (m, 3H), 7.03 (m, 1H), 5.58 (m, 1H), 5.11 (m, 5 H), 4.06 (m, 1H), 2.83 (m, 2H), 2.17 (m, 1H), 0.93 (m, 6H).

Example 30

2,6-Dichlorobenzyloxycarbonyl-Val-Asp-DCB-methylketone

Step A. *Z-Asp(OBu-t)-DCB-methylketone.* To a solution of Z-Asp(OBu-t)-bromomethylketone (500 mg, 1.24 mmol) in DMF (10 ml) was added potassian fluoride (320 mg, 5.50 mmol), and 2,6-dichlorobenzoic acid (348 mg, 1.82 mmol). The mixture was stirred at room temperature for 12 h, and then was diluted with 25 ml of ethyl acetate, washed with aqueous NH_4Cl and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained as white solid (0.78 g, 2.62 mmol, 69%). ^{1}H NMR ($CDCl_3$): 7.34 (m, 8 H), 5.96 (d, J = 8.7, 1H), 5.21 (d, J = 6.6, 2H), 5.16 (s, 2H), 4.70 (m, 1H), 2.88 (m, 2H), 1.27 (s, 9 H).

Step B. *Asp(OBu-t)-DCB-methylketone-N-hydrochloride.* To a solution of Z-Asp(OBu-t)-DCB-methylketone (572 mg, 1.14 mmol) in ethanol (15 ml) was added Pd/C (50 mg) and 6N HCl (0.2 ml). The mixture was stirred at room temperature under H_2 atmosphere (1 atm) for 12 h, then it was filtered and concentrated. The title compound was obtained as pale while solid (416 mg, 1.04 mmol, 90%). ^{1}H NMR ($CDCl_3$): 7.27 (m, 3H), 5.28 (m, 2H), 4.94 (m, 1H), 3.27 (m, 2H), 1.42 (s, 9 H).

Step C. *2,6-Dichlorobenzyloxycarbonyl-Val-Asp(OBu-t)-DCB-methylketone.* To a solution of 2,6-dichlorobenzyloxycarbonyl-Val (200 mg, 0.60 mmol) in THF (10 ml) was added Asp(OBu-t)-DCB-methylketone-N-hydrochloride (250 mg, 0.60 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 126 mg, 0.66 mmol), 1-hydroxybenzotriazole hydrate (HOBT, 92 mg, 0.60 mmol) and 4-(dimethylamino)pyridine (DMAP, 29 mg, 0.28 mmol). The resulting mixture was stirred at room temperature for 12 h, and diluted with ethyl acetate (20 ml), washed with 1N HCl, saturated $NaHCO_3$, brine and dried over Na_2SO_4. Evaporation of solvent, followed by flash chromatography (EtOAc/Hexane 2/3) gave the title compound as a colorless oil (85 mg, 0.18 mmol, 21%). ^1H NMR ($CDCl_3$): 7.35 (m, 6H), 7.27 (m, 1H), 5.41 (m, 3H), 5.15 (m, 2H), 4.41 (m, 1H), 2.96 (m, 2H), 2.28 (m, 1H), 1.44 (m, 9 H), 0.94 (m, 6H).

Step D. *2,6-Dichlorobenzyloxycarbonyl-Val-Asp-DCB-methylketone.* To a solution of 2,6-dichlorobenzyloxycarbonyl-Val-Asp(OBu-t)-DCB-methylketone (85 mg, 0.18 mmol) in CH_2Cl_2 (3 ml) at room temperature was added TFA (1 ml). The resulting solution was allowed to stir for 4 h, and then diluted with 20 ml of ethyl acetate, washed with saturated Na_2HPO_4, brine, and dried over Na_2SO_4. The solvent was evaporated in vacuo to give the title compound as a white solid (27 mg, 0.04 mmol, 25%). ^1H NMR ($CDCl_3$): 9.28 (bs, 1H), 8.11 (m, 1H), 7.49 (m, 1H), 7.30 (m, 6H), 5.37(m, 4H), 4.32 (m, 2H), 3.73 (m, 2H), 2.19 (m, 1H), 1.25 (m, 3H), 0.98 (m, 3H).

Example 31

Isobutoxycarbonyl-Val-Asp-fmk

The title compound was prepared in four-steps as described in Example 1 from L-valine and isobutyl chloroformate. ^1H NMR (DMSO-d_6): δ 8.47 (m, 1H), 7.23 (m, 1H), 5.24-4.52 (m, 3H), 3.81-3.72 (m, 3H), 2.73-2.55 (m, 2H), 1.94-1.79 (m, 2H), 0.89-0.83 (m, 12H).

Example 32

Methoxycarbonyl-Val-Asp-fmk

The title compound was prepared in four-steps as described in Example 1 from L-valine and methyl chloroformate. ^{1}H NMR (DMSO-d_6): δ 8.49 (s, 1H), 7.28 (m, 1H), 5.16-4.54 (m, 3H), 3.81 (m, 1H), 3.53 (s, 3H), 2.72-2.56 (m, 2H), 1.93 (m, 1H), 0.85 (m, 6H).

Example 33

Isopropyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in four-steps as described in Example 1 from L-valine and isopropyl chloroformate. ^{1}H NMR (DMSO-d_6): δ 8.43 (s, 1H), 7.11 (m, 1H), 5.18-4.54 (m, 4H), 3.76 (m, 1H), 2.69-2.67 (m, 2H), 1.91 (m, 1H), 1.17 (d, J = 6, 6H), 0.83 (m, 6H).

Example 34

Propionyl-Val-Asp-fmk

The title compound was prepared in four-steps as described in Example 1 from L-valine and propionyl chloride. ^{1}H NMR (DMSO-d_6): δ 8.63-7.82 (m, 2H), 5.37-5.02 (m, 1H), 4.70-3.95 (m, 3H), 2.89-2.56 (m, 2H), 2.21-2.10 (m, 2H), 1.91 (m, 1H), 0.98 (t, J = 7.2, 3H), 0.86-0.82 (m, 6H).

Example 35

Benzyl-glutaryl-Val-Asp-fmk

Step A. *Benzyl monoglutarate.* A solution of glutaric anhydride (460 mg, 4.03 mmol) in benzyl alcohol (1 ml) was heated at 70 °C overnight. The

mixture was purified by chromatography (3:1 hexane/EtOAc) to yield the product as an oil (0.4 g, 1.8 mmol, 45%). ^{1}H NMR ($CDCl_3$): δ 7.36 (br s, 5H), 5.12 (s, 2H), 2.48-2.41 (m, 4H), 2.00-1.96 (m, 2H).

Step B. *Benzyl-glutaryl-Val-OBu-t.* A mixture of benzyl monoglutarate (0.4 g, 1.8 mmol), Val-OBu-t (382 mg, 1.82 mmol), EDC (335 mg, 1.75 mmol), HOBT (265 mg, 1.73 mmol) and DMAP (372 mg, 3.04 mmol) in THF (15 ml) was stirred at room temperature for 17 h. It was worked up and purified by chromatography to yield the title compound as a colorless oil (370 mg, 0.98 mmol, 54%). ^{1}H NMR ($CDCl_3$): δ 7.39-7.30 (m, 5H), 5.96 (d, J = 8.4, 1H), 5.12 (s, 2H), 4.45 (m, 1H), 2.47-1.94 (m, 7H), 1.46 (s, 9H), 0.95-0.87 (m, 6H).

Step C. *Benzyl-glutaryl-Val-OH.* To a solution of benzyl-glutaryl-Val-OBu-t (370 mg, 0.98 mmol) in methylenechloride (3 ml) was added TFA (1 ml). The mixture was stirred at room temperature for 8 h. It was worked up to yield the title compound as a colorless oil (200 mg, 0.62 mmol, 63%). ^{1}H NMR ($CDCl_3$): δ 7.36 (br s, 5H), 6.43 (d, J = 8.7, 1H), 5.13 (s, 2H), 4.55 (m, 1H), 2.49-2.21 (m, 5H), 2.00 (m, 2H), 0.99-0.94 (m, 6H).

Step D. *Benzyl-glutaryl-Val-Asp-fmk.* The title compound was prepared from benzyl-glutaryl-Val-OH in three steps by a similar procedure as described in Example 1 as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.35 (br s, 5H), 6.72 (s, 1H), 5.56 (s, 1H), 5.11 (s, 2H), 4.92-4.22 (m, 4H), 3.13-2.68 (m, 2H), 2.43-1.93 (m, 6H), 0.92 (d, J = 5.1, 6H).

Example 36
Glutaryl-Val-Asp-fmk

A solution of benzyl-glutaryl-Val-Asp(OBu-t)-fmk (78 mg, 0.15 mmol) in 30% AcOH solution of HBr (2 ml) was stirred at room temperature for 4 h. The solvent was removed *in vacuo*. To the residue was added acetone (1 ml), then EtOAc (10 ml) and hexane (20 ml). The solvents were then

removed *in vacuo* to yield the title compound as a brown solid (36 mg, 0.10 mmol, 67%). ^{1}H NMR (DMSO-d_6): δ 8.60-7.87 (m, 2H), 5.36-4.42 (m, 4H), 2.94-2.57 (m, 2H), 2.33-1.69 (m, 7H), 0.83 (s, 6H).

Example 37

3-(2-Phenyloxyphenyl)propionyl-Val-Asp-fmk

Step A. *3-(2-Phenyloxyphenyl)propionic acid.* A mixture of 3-(2-hydroxyphenyl)propionic acid (0.57 g, 3.4 mmol), phenylbromide (0.4 ml, 3.37 mmol), and K_2CO_3 (2.5 g) in acetone (10 ml) was stirred at room temperature for 2 days. The mixture was diluted with 1:1 hexane/EtOAc (100 ml), washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by chromatography (5:1 then 4:1 hexane/EtOAc) to yield phenyl 3-(2-phenyloxyphenyl)propionate (0.45 g, 1.30 mmol, 77%).

A mixture of phenyl 3-(2-phenyloxyphenyl)propionate (0.45 g, 1.30 mmol), 2N NaOH (20 ml) and MeOH (10 ml) was stirred at room temperature overnight, which was then neutralized to pH~2 with concentrated HCl and extracted with EtOAc (100 ml). The organic phase was washed with brine and dried over sodium sulfate and concentrated *in vacuo* to yield 3-(2-phenyloxyphenyl)propionic acid as an oil. This was used for next step without further purification.

Step B. *3-(2-Phenyloxyphenyl)propionyl-Val-Asp-fmk.* The title compound was prepared in a similar procedure as described in Examples 36 and 1 from crude 3-(2-phenyloxyphenyl)propionic acid synthesized above. ^{1}H NMR (DMSO-d_6): δ 8.62-8.50 (m, 1H), 7.47-6.85 (m, 9H), 5.36-5.04 (m, 4H), 4.63-4.02 (m, 2H), 5.83 (br s, 6H), 1.85 (br s, 1H), 0.80 (s, 6H).

Example 38

3-(5-Bromo-2-hydroxyphenyl)propionyl-Val-Asp-fmk

A mixture of 3-(2-phenyloxyphenyl)propionyl-Val-Asp-fmk (30 mg, 30 mg, 0.06 mmol) in 30% acetic acid solution of HBr (2 ml) was stirred at room temperature for 6 h. The solvent was removed *in vacuo* and 1:1 EtOAc/hexane was added to the residue. Evaporation of the solvent yielded the title compound as a brown solid (18 mg, 0.037 mmol, 62%). ^{1}H NMR (acetone-d_6): δ 7.45-6.74 (m, 4H), 5.03-4.35 (m, 4H), 3.03-2.73 (m, 6H), 0.97-0.87 (m, 6H).

Example 39

3-Fluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3-fluorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.64 (m, 1H), 7.95 (m, 1H), 7.45 (m, 2H), 7.19 (m, 3H), 5.25 (m, 1H), 5.06 (s, 2H), 4.58 (m, 2H), 3.85 (m, 1H), 2.73 (m, 2H), 1.99 (m, 1H), 0.86 (m, 6H).

Example 40

2-Fluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 2-fluorobenzyl alcohol. ^{1}H NMR (DMSO-d_6): 7.36 (m, 2H), 7.12 (m, 3H), 5.68 (m, 1H), 5.17 (s, 2H), 4.34 (m, 3H), 3.97 (m, 2H), 2.62 (m, 2H), 2.06 (m, 1H), 0.91 (m, 6H).

Example 41

3-Methylbenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 1 from 3-methylbenzyl alcohol. ^{1}H NMR (DMSO-d_6): 8.52 (m, 1H), 7.92 (m, 1H), 7.43 (m, 1H), 7.17 (m, 4H), 5.25 (m, 1H), 4.99 (s, 2H), 4.56 (m, 2H), 3.83 (m, 1H), 2.71 (m, 2H), 2.29 (s, 3H), 1.93 (m, 1H), 0.85 (m, 6H).

Example 42

2-Chloro-4-fluorobenzyloxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 2 from 2-chloro-4-fluorobenzyl alcohol. ^{1}H NMR (CDCl3): 8.63 (m, 1H), 7.42 (m, 4H), 5.15 (m, 3H), 4.61 (m, 2H), 4.02 (bs, 1H), 3.81 (m, 1H), 2.63 (m, 2H), 1.90 (m, 1H), 0.83 (m, 6H).

Example 43

2-Naphthalenemethoxycarbonyl-Val-Asp-fmk

The title compound was prepared in five steps as described in Example 2 from 2-naphthalenemethanol. ^{1}H NMR (DMSO-d_6): 7.80 (m, 4H), 7.46 (m, 3H), 6.99 (m, 1H), 5.59 (m, 1H), 5.25 (s, 2H), 4.46 (m, 3H), 4.03 (m, 2H), 2.62 (m, 2H), 2.11(m, 1H), 0.94 (m, 6H).

Example 44

p-Toluenesulfonyl-Val-Asp-fmk

Step A. *p-Toluenesulfonyl-Val.* The title compound was prepared by a similar procedure as described in Example 1 step B in 13% yield from valine and p-toluenesulfonyl chloride. ^{1}H NMR (DMSO-d$_6$): 7.90 (d, J = 9.3, 1H),

7.63 (d, J = 8.1, 2H), 7.32 (d, J = 8.1, 2H), 3.47 (m, 1H), 2.35 (s, 3H), 1.90 (m, 1H), 0.82-0.76 (m, 6H).

Step B. *tert-Butyl 5-fluoro-3-[p-toluenesulfonyl -Valineamido]-4-hydroxypentanoate.* The title compound was prepared by a similar procedure as described in Example 1 step C in 37% yield. ^{1}H NMR ($CDCl_3$): 7.73 (d, J = 8.4, 2H), 7.30 (d, J = 7.2, 2H), 6.72-6.50 (m, 1H), 5.28-5.08 (m, 1H), 4.32-3.81 (m, 4H), 3.45 (m, 1H), 2.65-2.45(m, 2H), 2.43, 2.41 (2s, 3H), 2.05 (m, 1H), 1.45, 1.43 (2s, 9H), 0.88-0.79 (m, 6H).

Step C. *p-Toluenesulfonyl-Val-Asp(OBu-t)-fmk.* The title compound was prepared by a similar procedure as described in Example 1 step D in 92% yield. ^{1}H NMR ($CDCl_3$): 7.74-7.70 (m, 2H), 6.93 (m, 1H), 7.31-7.27 (m, 2H), 7.03 (d, J = 7.8, 1H), 6.96(d, J = 8.1, 1H), 5.26-4.61 (m, 3H), 3.55-3.47 (m, 1H), 2.98-2.48 (m, 2H), 2.11 (m, 1H), 2.43, 2.41 (2s, 3H), 1.45, 1.42 (2s, 9H), 0.87-0.81 (m, 6H).

Step D. *p-Toluenesulfonyl-Val-Asp-fmk.* The title compound was prepared by a similar procedure as described in Example 1 step E in 31% yield. ^{1}H NMR (DMSO-d_6): 8.53-8.43 (m, 1H), 7.81 (br s, 1H), 7.63 (d, J = 7.4, 2H), 7.32 (d, J = 7.4, 2H), 5.02-4.28 (m, 4H), 2.18-2.40 (m, 2H), 2.34 (s, 3H), 1.79 (m, 1H), 0.77-0.74 (m, 6H).

Example 45

p-Toluenesulfonyl-Phe-Asp-fmk

The title compound was prepared by a similar procedure as described in Example 1 in three steps from p-toluenesulfonyl-Phe and *t*-butyl 3-amino-5-fluoro-4-hydroxypentanoate. ^{1}H NMR (CD_3OD): 7.61 (d, J = 6.9, 2H), 7.29-7.13 (m, 7H), 4.56-3.94 (m, 4H), 3.01-2.78 (m, 2H), 2.44 (s, 3H), 2.51-2.37 (m, 2H).

Example 46
Enzyme Activity

The activity of 2-chlorobenzyloxycarbonyl-Val-Asp-fmk as an inhibitor of caspase-3 was measured in a fluorometric enzyme assay. Enzyme activity was measured using synthetic peptide substrates attached to a fluorogenic leaving group. Cleavage of the synthetic substrate by the enzyme results in a fluorescent signal which is read in a spectrofluorometer or in a fluorometric microtiter plate reader.

12 concentrations of the testing compound ranged from 30 pM to 10 μM were tested in the enzyme assay. The enzyme reaction was conducted in the presence of 2 ng rCaspase 3 (purchased from PharMingen, a Becton division company, San Diego, CA), various concentrations of testing compound, 10 μM caspase 3 substrate Ac-DEVD-AMC (SEQ ID NO:3) (purchased from Quality Controlled Biochemicals, Inc. Hopkinton, MA) and caspase buffer (20 mM PIPES, 100 mM NaCl, 10 mM DTT, 1mM EDTA, 0.1 % CHAPS and 10 % sucrose, pH 7.2) in a total volume of 100 μl. The enzyme reaction was carried out in a 96-well plate and incubated at 37 °C for 30 minutes. The plate was then read with a fluorescence plate reader (EG&G WALLAG 1420-002) using excitation filter at 355nm / emission filter at 460 nm. The data was analyzed using GraphPrism software. Other inhibitors were tested using the same procedure and the results is summarized in Table II.

Table II: Activity of Dipeptide as Inhibitor of Caspase-3

Name	Caspase-3 IC_{50} (nM)
2-Chlorobenzyloxycarbonyl-Val-Asp-fmk	36
3-Chlorobenzyloxycarbonyl-Val-Asp-fmk	36
Phenethoxycarbonyl-Val-Asp-fmk	110
4-Chlorobenzyloxycarbonyl-Val-Asp-fmk	34
Cyclohexylmethoxycarbonyl-Val-Asp-fmk	72
Ethoxycarbonyl-Val-Asp-fmk	58

As shown in Table II, the dipeptides are potent inhibitor of caspase-3.

Having now fully described this invention, it will be understood by those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any embodiment thereof. All patents, patent applications and publications cited herein are fully incorporated by reference herein in their entirety.